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Chemical Category	BENZENE 1,1'-METHYLENEBIS(ISOCYANATO- POLYMERS (26447-40-5)	

A 02

OFFICE OF TOXIC SUBSTANCES

REV. 7/27/82

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Chemical Name (300 per name) 25	CAS No. (10) 24

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A 03

INTERNATIONAL ISOCYANATE INSTITUTE, INC.

201 Main Street, Suite 403 • La Crosse, WI 54601 • 608/796-0880 • FAX 608/796-0882

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April 27, 1999

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Attn: 8(d) HEALTH 7 SAFETY STUDY REPORTING RULE
(REPORTING)

Dear Sir or Madam:

We herewith submit a copy of the following recently completed health and safety study.

"DIPHENYLMETHANE 4,4'-DIISOCYANATE (MDI-POLYMER):Evaluation of respiratory sensitization in guinea pigs following single high-level and repeated low-level inhalation induction exposure." A second report, "Guinea pig model for MDI asthma" is also enclosed-this details the determination of antibody levels for pr ct 11322, and is part of the overall study.

Name of Chemical Substance.	benzene 1,1'-methylenebis[iscoyanato-
Common name:	generic MDI
Chemical Abstracts Service Number:	26447-40-5
Abbreviation:	MDI

Contains No CBI

Authors:

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R. Dearman
Zeneca CTL
Macclesfield, Cheshire, UK

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The International Isocyat ate Institute (III) project identification number (11332) has been marked on the title page of the report. The supplemental report has been assigned (11323). Please refer to the III identification number(s) in any communication regarding this study. The enclosed report does not contain any Confidential Business Information.

This study was sponsored by the International Isocyanate Institute on behalf of the following:

The Dow Chemical Company
Bayer Corporation
BASF Corporation
ICI Americas, Inc.
Lyondell Chemical Company

Very truly yours,

M.J. Blankenship
Managing Director

Enclosure: Study

A 05

III Project 153 III ref. 11322 Volume 1

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1999 APR 30 AM 10: 52

Diphenylmethane 4,4'-diisocyanate (MDI-polymer) Evaluation of respiratory sensitisation in guinea-pigs following single high-level and repeated low-level inhalation induction exposure

J Pauluhn

Bayer AG
Department of Toxicology
Friedrich-Ebert-Str. 217-333
D-42096 Wuppertal
Germany

Scientific Office Note
This report describes and discusses results of a study which included determination of antibody titres, which are fully detailed in III Ref. 11323

Number of pages: 49

Contains No CBI

BAYER AG
DEPARTMENT OF TOXICOLOGY
FRIEDRICH-EBERT-STR. 217-333
D - 42096 WUPPERTAL

Report-No.: 28520

Date. 26.02.1999

Diphenylmethane-4,4'-diisocyanate (MDI-polymer)

Evaluation of Respiratory Sensitization in Guinea-pigs following single and repeated Inhalation Induction Exposure

by

PD Dr. J. Pauluhn

Study Number: T7062289

III-Project: 153 EU-MTX (PIP 271)

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R

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with the OECD Principles of Good Laboratory Practice (as revised in 1997) and to the Principles of Good Laboratory Practice (GLP) according to Appendix 1 German Chemicals Act (Bundesgesetzblatt Part I, July 29, 1994).

Date: June 8, 1998

PD Dr. J. Pauluhn D.A.B.T.
Board Approved Toxicologist (DGPT)
Study Director

STUDY MONITOR

Date: Oct 26/98

Dr. Timothy D. Landry D.A.B.T.
Toxicology Res. Lab., The Dow Chemical Co.,
Midland, MI 48674

SPONSOR

Date: November 4, 1998

International Isocyanate Institute, Inc.
Sponsor
International Isocyanate Institute, Inc.
201 Main Street, Suite 403
La Crosse, WI 54601
USA

1. TABLE OF CONTENTS

QUALITY ASSURANCE STATEMENT

Test Item: Diphenylmethane-4,4'-diisocyanate (polymeric MDI)

Study No.: T7062289

Study-based inspections/audits were conducted by the Quality Assurance on the dates given below. Audit reports have been submitted in writing to the study director and, if necessary, also the laboratory management, or other persons affected.

Date of audit		Date of report to study director and/or management
Jan. 20, 1998	(study plan)	Jan. 23, 1998
Jan. 28, 1998	(study conduct)	Jan. 28, 1998
Feb. 27, 1998	(study conduct)	Feb. 27, 1998
Mar. 20, 1998	(study conduct)	Mar. 23, 1998
Mar. 24, 1998	(study conduct)	Mar. 24, 1998
July 17, 1998 - Sep. 03, 1998	(first draft)	Sep. 07, 1998
Feb. 17, 1999	(final draft)	Feb. 17, 1999

The results of the study and the methods used have been correctly reported.

Quality Assurance Unit
PH-QA-C/GLP, Bayer AG

Date: Feb. 17, 1999

Responsible: [signature]
Dr.R.Rauchschwalbe

3. SIGNATURES

Study director: Dr. J. Pauluhn Jan. 29, 1999 Date

Analytical evaluation of test atmospheres: Dr. W. Rüngeler Feb. 4, 1999 Date

Head of department: Dr. E. Bomhard Feb. 25, 1999 Date

4. SUMMARY

A lung sensitization study with *polymeric* MDI (Desmodur® 44 V 20 L), subsequently abbreviated as PMDI, was performed using guinea pigs of the Hartley strain. This study used either a single or repeated inhalation exposure(s) for the sensitization of animals. Prior to sacrifice, animals were bled for IgG_1-anti MDI-antibody determinations.

Study design and exposure technology: Inhalation exposure of guinea pigs was made in directed-flow nose-only inhalation chambers to the aerosolized PMDI. Throughout the study, the aerosol demonstrated a highly respirable characteristic, i.e., the MMAD was approximately 1.5 µm, the GSD was ≈ 1.5 and there were no appreciable concentration-dependent effects on particle-size distributions. The targeted concentrations (see below) were met and confirmed by three independent analytical methods, i.e., the isocyana...-specific nitro-reagent method, by filter and cascade impactor analysis. All methods produced roughly identical results, thus demonstrating that the isocyanate functionality of PMDI was maintained after aerosolization.

Groups of ten female guinea pigs were either induced once on day 0 by a single inhalation exposure of 15-min (target concentration: 3, 10 and 30 mg PMDI/m³ air), 1-hr (target concentration: 3, 10 and 30 mg PMDI/m³ air) or 6-hrs (target concentration: 1, 3 and 10 mg PMDI/m³ air). Additional groups of animals were exposed for 6 hrs/day for 5 days/week on three consecutive weeks (target concentration: 1, 3 and 10 mg PMDI/m³ air). As the target concentrations were reasonably well met no differentiation between 'target' and 'actual' concentrations is made (for details *cf.* result section). Following a recovery period of approximately three weeks (single exposure) or shortly after the 3-week exposure period, animals of all groups were sacrificed and blood was collected for IgG1-anti MDI-antibody titer determinations (ELISA). The following additional end-points were considered: clinical observations, body weights and lung weights at sacrifice.

Summary of results: Following single and repeated inhalation induction, PMDI-exposed animals did not display any difference in clinical appearance from the respective air controls. Body weight gains were not markedly different from the concurrent control groups. Repeatedly exposed animals of 10 mg/m³ 3-week exposure group showed a decrease in body weights gain towards the end of study. In this group the absolute and relative lung weights were statistically significantly increased whilst the remaining groups appeared to be indistinguishable from the concurrent control group.

Animals in the single-exposure MDI-induction groups elaborated concentration x time-dependent IgG_1-anti MDI-antibody titers. In the 3-week repeated exposure inhalation study no such relationship could be established (ostensibly the maximum response was observed in all MDI-exposure groups). IgG_1-anti MDI-antibody was not detectable in any of the control animals. In the repeated exposure study, there was gross pathologic indication of pneumotoxicity in guinea pigs exposed to 10 mg/m^3, however, the IgG_1-anti MDI-antibody did not appear to correspond with this response. As illustrated by the analytical characterization of test atmospheres as well as by the continuous real-time monitoring of atmospheres, there were no apparent short-term peak excursions in exposure concentrations in the 1 and 3 mg/m^3 groups.

Taking into account the intensity and duration of exposure, serological data show some concentration x time relationship. It appears, however, that high(er) concentrations during a short period of time are apparently more critical for IgG_1-anti MDI-antibody induction than lower concentrations during a longer period of time. This means, despite increased cumulative dose, there is a lack of a proportional increase in antibody production. The comparison of exposure concentrations with the respective cumulative concentration x time relationships appears to suggest that IgG_1-anti MDI-antibody production is a saturable process and that for the repeated exposure regimen the maximum response was apparently attained in all MDI-exposure groups. However, one major difference of the single and repeated exposure regimens is that the animals were sacrificed after a 3-week postexposure period and 1-day after the last exposure, respectively. If one would consider the repeated exposure to be also a possible re-challenge type of exposure, then IgG_1-anti MDI-antibodies may have been sequestered at the location of first contact with the inciting agent, viz., in the respiratory tract. Therefore, due to the absence of any re-challenge type of exposure, the results obtained by single and repeated exposures cannot directly be compared, since antibody levels in the peripheral blood may not necessarily reflect those of the lung.

5. INTRODUCTION

Guinea pigs are known to produce high titers of anti-MDI IgG_1-antibodies and current knowledge suggests that determination antibody titers appear to indicate the extend and duration of exposure most reliably. It appears to be generally accepted that an IgG_1 antibody response provides the potential for the elicitation of a pulmonary response to occur; however, there is no clear relationship between antibody titer and pulmonary responsiveness or the severity of any pulmonary response in guinea pigs or humans (Blaikie *et al.*, 1995; Pauluhn and Eben, 1991; Lushniak *et al.*, 1998). Therefore, the objective of this study is to analyze the dependence on the exposure protocol (short-term high level *versus* repeated low-level exposure) for the induction of IgG_1 antibody response as marker of exposure.

Briefly, the induction of an immunological response is assessed by the determination of IgG_1-anti-MDI antibody using an ELISA assay following single inhalation exposures of 15-min, 1- and 6-hr to various MDI-concentrations (1 - 30 mg/m^3 air). Guinea pigs are sacrificed about day 21 following the first induction exposure and blood is collected for antibody determinations. During the 1-hr exposure regimen, measurements of the respiratory minute volume are made to allow a more precise calculation of 'dose'. In a second study guinea pigs are exposed for 6-hr/day, on 5 consecutive days/week for 3-weeks. At the end of study, blood is collected for antibody determinations.

Testing facility:

Institute of Toxicology - Industrial Chemicals/Section Occupational Toxicology, Bayer AG, D-42096 Wuppertal, Friedrich-Ebert-Straße 217 - 333, Germany.

Sponsor:
International Isocyanate Institute, Inc.
201 Main Street, Suite 403
La Crosse, WI 54601
USA

Study/project identification:
Study no.: T7062289 / III-project PIP 271 - 153 EU-MTX

Start of study: January 26, 1998
Experimental starting date: January 19, 1998 (technical pre-trials)
Adaptation of acutely exposed animals (3 pre-exposures) January 21, 1998
End of study: M rch 20, 1998

6. RESPONSIBILITIES

Air conditioning, purification .... Dipl. Ing. G. Strietholt
Biometrical evaluation & software development ....Dr. J. Pauluhn
Characterization of test atmospheres ....Dr. W. Rüngeler
Experimental Animal Services Office ....Dr. Petersen v. Gehr
Archiving of study data ....Prof. G. Schlüter
Necropsy .... Dr. M. Rosenbruch
Quality Assurance .... Dr. H. Lehn
Serological determinations .... Dr. R. Dearman/ZENECA
Study director ....Dr. J. Pauluhn
Study monitor ....Dr. T.D.Landry/DOW CHEMICAL, U.S.A.
Test compound supply & coordination .... Dr. Pilger/BAYER AG

7. MATERIAL AND METHODS

7.1. Test Substance

Chemical name: Diphenylmethane-4,4'-diisocyanate (MDI-polymer)

Abbreviation: PMDI

Commercial name: DESMODUR® 44 V 20 L[1]

Manufacturer: BAYER AG, Leverkusen, Germany

General specification: (*not documented in* raw data)
≈ 50% *monomeric* 4,4'-MDI
≈ 4% *monomeric* 2,4'-MDI
≈ 34% 3-*oligomeric* MDI
≈ 9% 4-*oligomeric* MDI (balance: higher *oligomers*)

Batch no.: 7920/L2D

Storage conditions: refrigerator (≈ 4 °C) / darkness {prior to study}

Storage conditions: at room temperature / darkness {during study}.

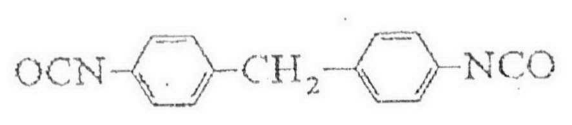
Handling: complete exclusion of air/humidity (handling and storage in dry nitrogen)

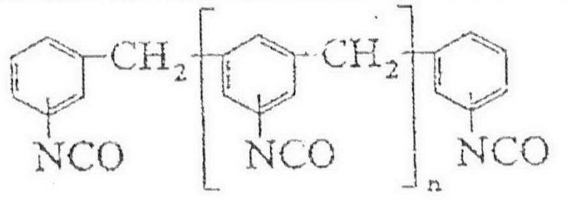
Appearance: brownish, translucent liquid material (viscous)

Empirical formula (*of monomer*): $C_{15}H_{10}N_2O_2$

Molecular weight: 250.3 g/mol (monomer)

Representation of *monomeric* (left panel) and *polymeric* MDI (right panel) in their generic forms:





[1] DESMODUR is a trade name of BAYER AG, Leverkusen, Germany. PMDI of other producers differ only slightly in chemical and physicochemical properties.

7.2. Test System and Animal Maintenance

Species and rationale: The study was conducted with female guinea pigs - an animal species recommended for lung sensitization studies.

Young adult, healthy pure-bred guinea pigs of the Hartley strain [Crl:(HA)BR] from the Charles River Wiga GmbH Experimental Animal Breeders in Sulzbach (Germany) were used. This strain of animals has also been used for validation studies at Bayer. Historical data on the physiology are available. The state of health of the breed is monitored and the animals are routinely spot-checked for the primary specific pathogens. The results of these tests are retained.

Acclimatization: The animals were acclimatized to the animal room conditions for at least five days before use except for controls [single exposure: date of receipt January 20, 1998, entry no.: 94467; repeated exposure: date of receipt January 24 and 27, 1998, entry nos.: 94478 and 94470]. Animals of the single-exposure groups were acclimatized to the exposure restrainers for 3 days.

Identification: Animals were identified by both individual color-marking and cage-labels. All animals from this study were located on labeled cage-racks.

Randomization: Before the start of the study the health status of each animal was assessed. Animals were subsequently assigned to exposure groups at random (the randomization procedure is described in the statistics section).

Health status: Only healthy animals free of clinical signs were used for this study. The animals were not vaccinated or treated with anti-infective agents either before their arrival or during the acclimatization or study periods.

Age and weight: At the study start the variation of individual weights did not exceed ± 10 per cent of the mean (see Appendix). Animals of the weight class used were approximately 2 months old.

Animal housing: During the acclimatization and study periods four animals per cage were housed under conventional conditions in conventional Makrolon® Type IV cages (based on A. Spiegel and R. Gönnert, Zschr. Versuchstierkunde, 1, 38 (1961) and G. Meister, Zschr. Versuchstierkunde, 7, 144-153 (1965)). Cages bottles were changed twice a week while unconsumed feed and water were changed once per week. The legal requirements for housing experimental animals (86/609 EEC) were followed.

Bedding: Bedding consisted of type S 8/15 low-dust wood granulate from Ssniff, Soest/Westfalen, Germany. The wood granulate was randomly checked for harmful constituents at the request of the Central Animal Supply Department, Bayer AG.

Animal rooms: All animals were housed in a single room.

Environmental conditions in the animal room

The animal room environment was as follows:

Room temperature:	22 ± 2 °C
Relative humidity:	approximately 50 %
Dark/light cycle:	12 h/12 h; artificial light from 6.00 a.m. to 6.00 p.m. Central European Time
Light intensity:	approximately 14 watt/m² floor area
Ventilation:	approximately 10 air changes per hour

The room humidity and temperature were continuously monitored and documented using a calibrated thermohygrograph. Occasional deviations from these conditions occurred, e.g. as a result of animal room cleaning, but these had no detectable influence on the outcome of this study.

Cleaning, disinfection, and pest control: The animal room was regularly cleaned and disinfected once a week with an aqueous solution of TEGO® 2000. Contamination of the feed and contact with the test system were excluded. Pest control was not conducted in the animal room.

Feeding: Rations consisted of a standard fixed-formula diet (Altromin® 3022 maintenance diet for Guinea pigs, Altromin GmbH, Lage) and tap water (drinking bottles). Both food and water were available *ad libitum*. The pelletized feed was contained in a rack in the stainless-steel wire cage cover.

The nutritive composition and contaminant content of the standard diet were checked regularly by random sampling by the Central Animal Supply Department, Bayer AG. Details concerning general feed and water specifications are provided in the Appendix.

Water: Drinking quality tap-water (Drinking Water Decree of 05.12.1990, Bundesgesetzblatt [federal law gazette] part I, page 2612) was provided *ad libitum* in polycarbonate bottles containing approximately 700 ml (based on A. Spiegel and R. Gönnert, Zschr. Versuchstierkunde, 1, 38 (1961) and G. Meister, Zschr. Versuchstierkunde, 7, 144-153 (1965)). The results of feed and water analyses are retained by Bayer AG. The available data provided no evidence of an impact on the study objective.

7.3. Test Guidelines

The technical exposure criteria specified in OECD Guideline No. 403 and the corresponding EC Guideline 84/449/EWG were fulfilled insofar as these were applicable to this study. Other recommendations (US EPA, 1988) were also considered so as to comply with internationally recognized procedures. Specific, internationally harmonized test procedures for experiments to assess the development of lung sensitization in appropriate animal models do not currently exist.

7.4. Study Design

Each group consisting of 10 female guinea pigs was exposed as shown below. Animals subjected to a single exposure were acclimatized to the restraining tubes prior to exposure to PMDI (1 hr/day on 3 consecutive days) in order to reduce possible stress related variability on ventilation and, accordingly, in dosimetry.

Group allocations: Specific information regarding the group allocation and challenge exposed is provided in the following summary table.

By **single inhalation exposure:**

I (*day 0, 1x15 min; nose only exposure):* Controls are exposed to conditioned air only. Target concentrations: 3, 10, and 30 mg polymeric MDI/m³ air.

II (*day 0, 1 x 1-hr; nose only exposure):* Controls are exposed to conditioned air only. Target concentrations: 3, 10, and 30 mg polymeric MDI/m³ air. During exposure the respiratory minute volume is determined.

III (*day 0, 1 x 6-hr; nose only exposure):* Controls are exposed to conditioned air only. Target concentrations: 1, 3, and 10 mg polymeric MDI/m³ air.

By **repeated inhalation exposure:**

IV (*6-hr/day 5 time/week for 3 consecutive weeks; nose only exposure):* Controls are exposed to conditioned air only. Target concentrations: 1, 3, and 10 mg polymeric MDI/m³ air. Simultaneous exposure of ten female guinea pigs per concentration.

Specific endpoints:

Respiratory parameters during exposure (1 x 1 hr regimen only): Base-line data are collected during a 15-min pre-exposure period to air followed by a 1-hr exposure period to PMDI. Recovery data are collected during a 30-min post-exposure period to air.

MDI-specific IgG_1 antibody titer: days 21 or 22

Organ weights: Lung weights

Observations and body weights: Clinical observations will be performed at least once a day (before and after exposure). Body weights are recorded on days 0, 3, 7, and weekly thereafter and before sacrifice in the single-exposure study and weekly in the repeated exposure study.

Serological evaluation: During sacrifice of guinea pigs sera are collected for immunological assessment.

Exposure Regimen

Group	N	Exposure Duration	Target Exposure Concentration (mg PMD/m³ air)	IgG_1-Antibodies
1	10	1 x 15-min	0	day 21 / 22
2	10	1 x 15-min	3	day 21 / 22
3	10	1 x 15-min	10	day 21 / 22
4	10	1 x 15-min	30	day 21 / 22
5	10	1 x 1-hr	0	day 21 / 22
6	10	1 x 1-hr	3	day 21 / 22
7	10	1 x 1-hr	10	day 21 / 22
8	10	1 x 1-hr	30	day 21 / 22
9	10	1 x 6-hr	0	day 21 / 22
10	10	1 x 6-hr	1	day 21 / 22
11	10	1 x 6-hr	3	day 21 / 22
12	10	1 x 6-hr	10	day 21 / 22
13	10	15 x 6-hr	0	day 21 / 22
14	10	15 x 6-hr	1	day 21 / 22
15	10	15 x 6-hr	3	day 21 / 22
16	10	15 x 6-hr	10	day 21 / 22

7.5. MDI-Exposure Technique

Mode of exposure: Animals were exposed to the aerosolized test substance in restrainers made of Plexiglas. Restrainer tubes were chosen that accommodated the animal's size. The design of the *directed-flow* inhalation chamber prevents re-breathing of the test atmosphere (Moss and Asgharian, 1994). This type of exposure is preferable to whole-body exposure on scientific (Pauluhn, 1984) and technical reasons (rapid attainment of steady-state concentrations, no problems with regard to test atmosphere inhomogeneities, better capabilities to control all inhalation chamber parameters, easier cleaning of exhaust air, and lower consumption of test substance). Moreover, contamination of the hair-coat can largely be avoided. The operation of this commercially available chamber (TSE company in Bad Homburg v.d.H., Germany) and its validation has been published in detail (Pauluhn, 1994).

Generation of atmosphere: Atmospheres of PMDI for inhalation exposures were generated under dynamic conditions using a digitally controlled Hamilton Microlab M pump and a modified Schlick-nozzle Type 970, form-S 3 (Schlick GmbH, Coburg, Germany). The test substance was nebulized using conditioned (dry, oil-free) compressed air (dispersion pressure approximately 600 kPa). The liquid containing parts of the nozzle were kept at approximately 40 °C by a water jacket connected to a digitally controlled JULABO thermostat. The increase of temperature within the nozzle resulted in a marked decrease in viscosity and hence increased reproducibly the output of aerosol. The respective concentration was achieved by applying the extraction/dilution cascades as depicted in Fig. 1. Finally, prior to entering the inhalation chamber, the level of PMDI aerosol was adjusted with additional dilution flows of conditioned air so as summarized in Table 1 (see result section).

Inhalation Chamber: Each segment of the aluminum inhalation chamber has the following dimensions: inner diameter = 14 cm, outer diameter = 35 cm (two-chamber system), height = 25 cm (internal volume = about 3.8 l). The construction of the inhalation chamber is shown schematically in Fig. 1. For this study a two segment-chamber was used.

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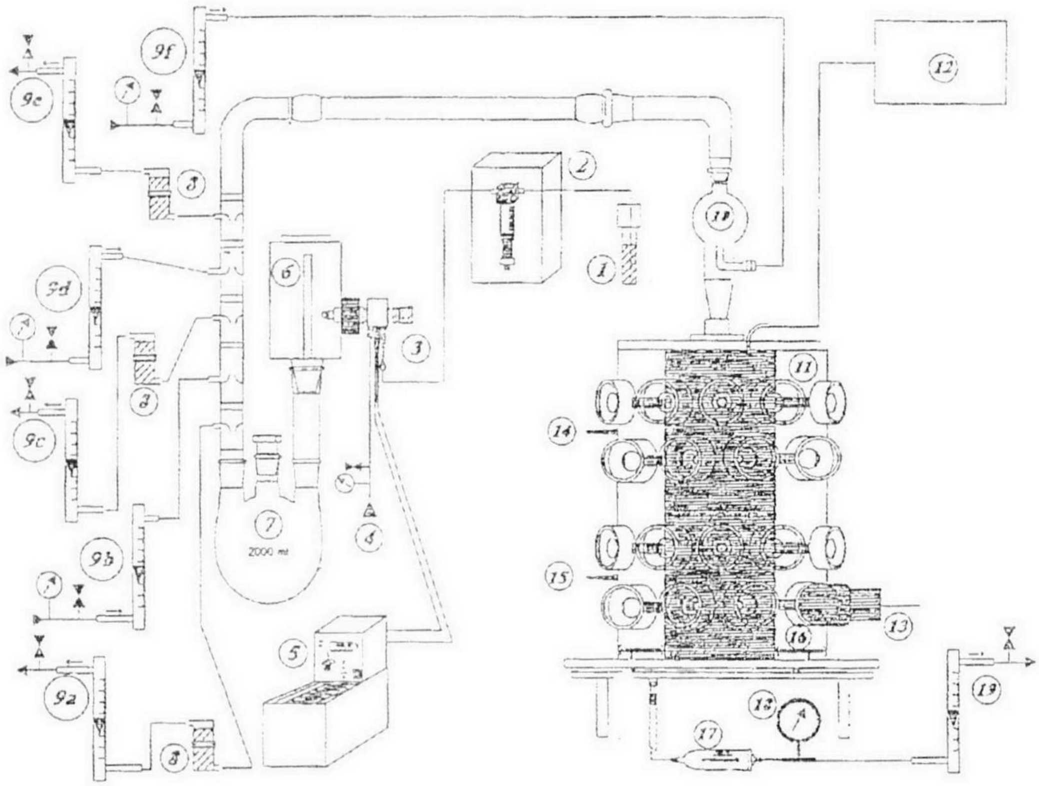
Figure 1: Inhalation chamber (schematic)



1. PMDI-reservoir 2. Digital Microlab pump 3. Schlick-nozzle with water jacket @ 40°C 4. Conditioned pressurized air 5. Digitally controlled thermostat 6. Baffle / pre-separator 7. Expansion / settling chamber 8. Filter (for air extracted) 9. Flow-meter to control subtracted / added air-flows a,b) 1st dilution unit c,d) 2nd dilution unit e,f) 3rd dilution unit	10. Cyclone and final dilution of PMDI atmosphere 11. Directed-flow nose-only exposure zone 12. Aerosol photometer (*real-time* monitoring) 13. Sensor for temperature and humidity measurement 14,15. Sampling location ('breathing zone sampling') 16. Exhaust location of exhaled atmosphere 17. HEPA-filter 18. Pressure gauge 19. Flow-meter followed by make-up of exhausted atmosphere (Cotton-wool aerosol filter + HEPA filter)

Dilution of atmosphere: The objective of this study was to generate different concentrations of adequately respirable PMDI aerosol without marked concentration-

4

dependent changes on particle-size distribution. This has been achieved by dilution of the PMDI aerosol using extraction/dilution cascades rather than by change of the principle aerosolization process. The respective dilution ratios are summarized in Table 1 (see result section).

Compressed air conditioning: The compressed air was produced with two Boge Model SB 270/15/350D compressors operated in parallel. The air was automatically conditioned (i.e. water, dust and oil removed) by subsequent passa through a VIA compressed air dryer. The regulated operating pressure of the co ssors was 8 - 10 bars (800 - 1000 kPa). Pressure-reduction valves were used to set the operating pressure.

Inhalation chamber - steady-state concentration: The test a sphere generation conditions assured at least 230 air volume exchanges per hour. steady state was established in less than approximately one minute of operatio nder these test conditions ($t_{95\%}$ = 3 x chamber volume/air flow rate; McFarland, 1976). The ratio of input to exhaust air was selected to ensure that approximately 80-90% of the input air was removed by the exhaust system, and the remainder via other chamber openings. An air flow towards the guinea pigs' exposure zones was thus provided in the exposure system (*directed-flow* principle) allowing an adequate ventilation of the animals' breathing zone.

Air flows: Air flows are monitored and controlled continuously by a calibrated precision flowmeters of the Fischer & Porter company. For calibration purposes the "generic" scale of the tapered flow-meter is derived mathematically taking into account the current ambient pressure and temperature (software supplied by Fischer & Porter, Göttingen, Germany). To ensure proper calibration, the mathematically derived scale is confirmed by soap bubble meter measurements (GILIBRATOR, Ströhlein Instruments, Kaarst, Germany). Flow-meters are always used between 25% and 75% of their capacity. Also the calibration of mass flow controllers is performed using the GILIBRATOR.

Repeated inhalation exposure: For the repeated inhalation study, a more computerized exposure technology was used. Briefly, again dry conditioned air was used to aerosolize the PMDI so as described above. All air flows are monitored and adjusted continuously by means of calibrated and computer controlled mass-flow-controllers. A soap bubble meter was used to monitor the accuracy of mass-flow-controller. As demonstrated in Table 1, the ratio between supply and exhaust air was selected so that 90% of the supplied air was extracted via the exhaust air location and, if applicable, via sampling ports. HEPA-filters was used for exhaust air clean-up. During sampling, the exhaust air was reduced in accordance with the sampling flow rate using a computerized HP 3852A Data Acquisition and Control System so that the total exhaust air flow rate was adjusted on-line and maintained at the specified

90%. The slight positive balance between the air volume supplied and extracted ensured that no passive influx of air into the exposure chamber occurred (via exposure restrainers or other apertures). The slight positive balance provides also adequate dead-space ventilation of the exposure restrainers. The pressure difference between the inner inhalation chamber and the exposure zone was 0.02 cm H_2O (Pauluhn, 1994). The exposure system was accommodated in an adequately ventilated enclosure. Temperature and humidity are measured by the HP 3852A Data Acquisition and Control System using calibrated sensors. The sensors were located at the exposure location of the inhalation chamber (*cf.* Fig. 1). Further technical details are provided in the ensuing sections.

Air flows/repeated exposure: Air flows are monitored and controlled continuously by calibrated mass flow meters (Hastings HFC-C Mass Flow Controllers, Teledyne Hastings-Raydist, Hampton, VA, USA). For analytical sampling TYLAN FC-280 S mass flow controller are used (TYLAN General, Torrance, California, USA). The calibration of mass flow controllers is performed by computer under actual operating conditions. Voltage specifications exceeding or falling below the specified range are indicated by an alarm/error listing. The Data Acquisition and Control System monitors/controls up to five inhalation chambers simultaneously.

Computer control technique/repeated exposure: The process control system (PCS) establishes a secured PC-internal study protocol (HP Vectra QS/25) which determines all basic physical inhalation chamber operating parameters for the study. Non adherence to the specifications are indicated during the study by an alarm (acoustical and optical). The PCS continuously monitors, controls, and/or records the inhalation chamber parameters: supply and exhaust air, all sampling activities, real-time aerosol monitoring, temperature and humidity. The PCS also documents the exact daily duration of exposure, and files all individual sampling data (i.e. time, date, sample no., chamber no., study no., flow rate, integrated volume, chamber temperature as well as the corresponding analytically determined concentrations). The PCS manages the historical and actual sensor calibration data, and after each re-calibration of a particular sensor, drifts or sensor instabilities are analyzed. Control of the inhalation chamber and management of all physical inhalation chamber data, including the current calibration data, are performed using a HP 3852A Data Acquisition and Control System. The equipment uses integrated voltmeter with automatic zero balance (HP 44701A), one 20-channel relay multiplexer (HP 44705A), and HP 44727A digital/analog converters. An HP Vectra QS/25 computer is used for evaluation and control. The measurement, control of sensors and mass flow meters, and the data acquisition are supported by the HP software PCATS.

Exhaust air treatment: The exhaust air was purified by passage through a series of aerosol filters (1. cotton wool filter, 2. HEPA filter). The filters were destroyed by incineration in appropriate Bayer AG facilities.

Inhalation exposure - occupational hygiene: Contact with or the potential of exposure of the operator was minimized and was accomplished by placing the exposure system into a horizontally ventilated enclosure (chemical fume hood). A negative pressure gradient between the enclosure and laboratory prevented any outward leakage from the enclosure. The temperature in the laboratory accommodating the enclosures was kept at 22 ± 1 °C.

7.6. Inhalation chamber temperature and humidity

Single exposure: Temperature and humidity values were determined using the Leybold-Heraeus system as described below. The sensor was located in the vicinity of the breathing zone as shown in Fig. 1. Readings were recorded at 10-minute intervals. The humidity-detecting cell was protected against aerosols by a Teflon® membrane (pore size about 1 μm) sandwiched between two sintered-metal filters. Readings were transmitted through an IEEE 488 interface and recorded and analyzed using an Apple IIe computer equipped with an MDP 8240/45 analog/digital converter. Sensors were calibrated as described below.

Repeated exposure: Temperature and humidity measurements are also performed by the computerized HP 3852A Data Acquisition and Control System using FTF11 sensors (ALKA ELEKTRONIK, Lüdenscheid, Germany). The position of the measuring probe was at the exposure location of guinea pigs (*cf.* Fig. 1). Measurements were performed predominantly in the lower segment. Temperature and humidity data are integrated for 30-seconds and displayed accordingly. The humidity sensors are calibrated using saturated salt solutions according to Greenspan (1977) and Pauluhn (1994) in a two-point calibration at 33% ($MgCl_2$) and at 75% (NaCl) relative humidity. The calibration of the temperature sensors is also checked at two temperatures using reference thermometers.

7.7. Analytical Characterization of Test Atmosphere

The nominal concentration was calculated from the ratio of the quantity of test substance sprayed into the baffle and the total throughput of air through the inhalation chamber. Specific information concerning air-flows and test atmosphere

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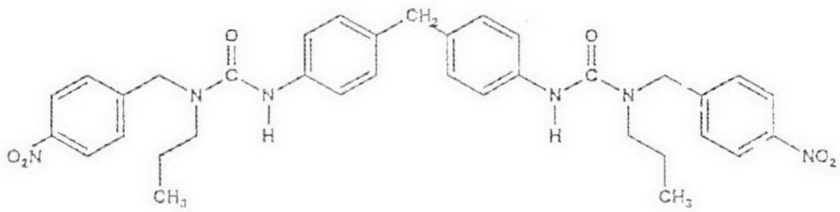
concentrations are provided in Table 1. The lower analytical concentrations compared with the nominal concentrations are attributed to the efficient removal of larger particles in the baffle/preseparator system.

Gravimetric evaluation: The test-substance concentration was determined by gravimetric analysis (filter: Glasfibre-Filter, Sartorius, Göttingen, Germany; balance: Mettler AE 100).

Analytical evaluation: The test-substance concentration was determined using the methodology described in the Appendix (Analytical Report). The breathing zone samples of PMDI were taken from the chamber atmosphere using two in-line connected tubes packed with glass-powder-filled sampling tubes containing N-4-nitrobenzyl-N-n-propylamine as a scavenger for intact PMDI, according to the method published by Dunlap *et al.* (1976). The resultant urea derivative was subsequently extracted using acetonitrile (Baker, HPLC Gradient Grade) and analyzed by high performance liquid chromatography (HPLC). The reported concentrations of PMDI are based on nitroreagent determinations when not otherwise specified.

Nitroreagent-MDI-Urea-Derivative (generic formula)



A minimum of one (single exposure) or three (repeated exposure) representative samples of PMDI atmospheres were taken from the inhalation chamber (cf. Figure 1) per exposure. The flow rate during sampling was 1 liter/minute for nitroreagent analysis. Sampling for gravimetric analyses was 4 liters/minute. Gravimetric analyses were performed prior to exposure of animals and served the purpose of 'fine tuning' of the aerosol generator.

7.8. Stability of Test Atmosphere

The stability of the aerosol generation system was checked using a RAS-2 aerosol photometer (MIE, Bedford, Massachusetts, USA). Samples were taken continuously from the vicinity of the animals' breathing zone. This chamber monitoring allows for an overall survey of toxicologically relevant technical parameters (inlet and exhaust flows as well as atmosphere homogeneity, temporal stability, and generation performance). Hence interruptions in exposure (e.g. resulting from obstruction of the nozzle or other technical mishaps) could be recorded appropriately, if applicable.

7.9. Test atmosphere Particle Characterization

Samples for analysis of particle-size distribution were also taken in the vicinity of the breathing zone. These samples were taken using a low-pressure critical orifice cascade impactor. Specifications and representative example evaluations are provided in the Appendix. The individual impactor stages were covered with aluminum foils which had been evaluated by gravimetric analysis. Due to the adhesive properties of the test compound a coating for these surfaces was not considered to be necessary (to prevent particle bounce).

Evaluation of particle-size distributions

For the evaluation of the cascade impactor analyses the mass median aerodynamic diameter (MMAD) and the geometric standard deviation (GSD) were determined from the probit-transformed cumulative particle mass frequency distribution (y-axis) and the logarithmic effective cut-off diameters (ECD's) (x-axis) of the individual impactor stages by linear regression. The GSD was calculated from the regression line: percentile 84 / percentile 50. The relative mass with an aerodynamic diameter ≤ 3 µm (*"respirable mass fraction"*) [Raabe, 1982; Snipes, 1989; SOT-Commentary, 1992] was calculated from the regression line. For probit transformation and linear regression FORTRAN algorithms were used.

To verify whether the aerosol distribution was in fact monomodal and log-normal the normalized mass per stage (f_H') was evaluated as a histogram. $\Delta \log D_p$ is equal to the difference $\log D_{p+1} - \log D_p$, whereas D_p is the lower (left) cut-size limit and D_{p+1} the higher (right) cut-size limit of the corresponding impactor stage. As demonstrated by the evaluations included in the Appendix, the impactor stage cut-off limit (D_{p+1}) to the right was used for all calculations.

$$f_H' = \frac{1}{N_f} \times \frac{mass\ /\ stage}{\Delta \log D_p}$$

The log-normal mass distribution $y'(D_{ae}) = 1/N_f \times y(D_{ae})$ as a function of the aerodynamic diameter (D_{ae}) was computed using the formula:

$$y'(D_{ae}) = \exp\left[-\frac{(\log D_{ae} - \log MMAD)^2}{2 \times \log^2 GSD}\right]$$

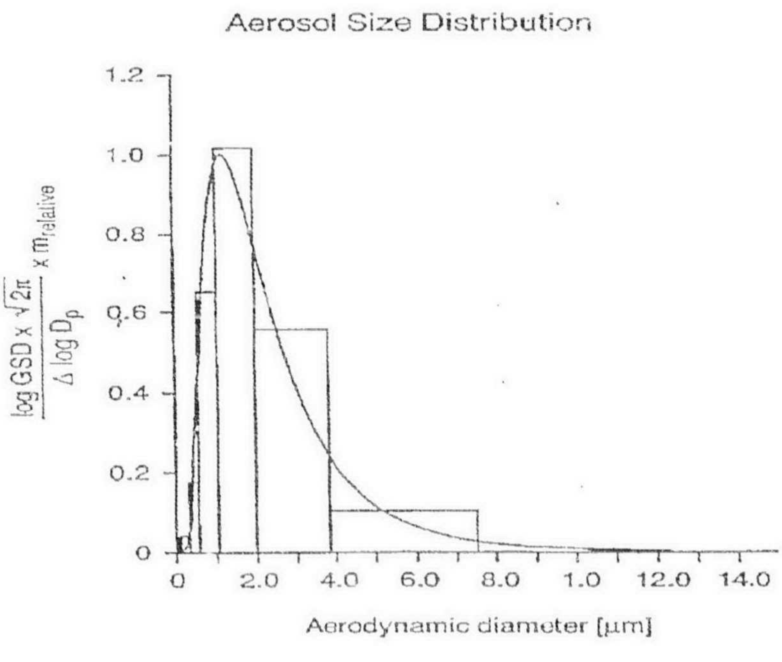
The normalization factor (N_f) was calculated as follows:

$$N_f = \frac{\Sigma mass}{\log GSD \times \sqrt{2\pi}}$$

where Σmass was the total mass collected by the cascade impactor, and $m_{relative}$ = mass per stage/Σmass (Fig. 2).

The algorithm for the calculation of particle size characteristics was taken from pertinent reference works on aerosol physics (Dennis, 1976; Marple and Rubow, 1980) and has proved to be generally applicable (Pauluhn, 1994).

Figure 2: Principle of evaluation of particle-size distribution



The particular advantage of this type of evaluation is that the calculated particle-size distribution can directly be compared with the corresponding raw data in order to assess *visually* the quality of the fit and whether the distribution is indeed monomodal and log-normal.

Respirability: The particle mass smaller than 3 µm was considered to be respirable for the guinea pig (Raabe, 1988).

7.10. Collection Efficiency

The sampling equipment was adjusted with calibrated flow meters or soap bubble meters according to internationally recognized standards (ACGIH, 1978; Section I "Calibration of Air Sampling Instruments"). Sampling of atmosphere was performed from the inner cylinder of the inhalation chamber (see Fig. 1). Concentrations obtained at this location are representative for *"breathing zone samples"* (*cf.* Pauluhn, 1994).

The conditions for test atmosphere generation were optimized to provide maximum aerosol respirability to guinea pigs (Raabe, 1982; Snipes, 1989; SOT-Commentary, 1992). The absence of larger particles and high flow rates in the vicinity of the sampling ports make it possible to disregard potential anisokinetic sampling errors, thus ensuring a representative sampling even with different sampling probe orifice diameters and flow rates. The tolerance limits for the radius of the probe orifice were calculated using the following formula [ACGIH, 1978]. Calculations consider both a particle size distribution that encompasses aerodynamic diameters (D_{ae}) of 0.5 to 7.4 µm and sample flows ranging from 8 to 80 ml/sec.

$$\boxed{5 \times \sqrt[3]{\frac{flow \times \tau}{4 \times \pi}} \leq r_p \leq \frac{1}{5} \times \sqrt[2]{\frac{flow}{g \times \tau \times \pi}}}$$

r_p = radius of the sample probe in cm = ½ x D_p
τ = relaxation time ($D_{ae\ 0.5\ \mu m}$ = 1×10^{-6} sec; $D_{ae\ 7.4\ \mu m}$ = 1.7×10^{-4} sec)
g = gravity constant = 980 cm/sec^2

Tolerance limits calculations for the sample probe orifice (r_p) indicated that a representative sampling was assured when the orifice inner diameter was in the range of 1.0 to 1.6 cm. Orifices of the sampling instruments used here were consistent with this criteria. Details of the D_p tolerance limit calculations are published elsewhere (Pauluhn, 1988; Pauluhn, 1994).

7.11. Body Weights and Observation Period

The body weights were determined prior to induction, on relative study days three and seven, and weekly thereafter (single exposure). In the repeated exposure study, body weights were determined repeatedly during the course of study (for details see Appendix). Animals were also weighed before necropsy.

7.12. Clinical Signs

The appearance and behavior of each guinea pig was examined carefully several times on the day of exposure and once daily thereafter (including weekends). Animals of the repeated exposure regimen were observed twice daily, before and after exposure and once daily during weekends. Assessments from restraining tubes were made only if unequivocal signs occurred (e.g. spasms, abnormal movements, severe respiratory signs). Following exposure, observations were made and recorded systematically; individual records were maintained for each animal. Cage-side observations included, but were not limited to, changes in the skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system, and somatomotor activity and behavior pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhea, lethargy, somnolence and prostration.

7.13. Respiratory Function Measurements

Measurements were conducted with spontaneously breathing, conscious guinea pigs of the 1 x 1 hr exposure group in modified nose-only exposure tubes used as plethysmographs. The animals were acclimatized to the exposure conditions for an adequate period of time.

After acclimatization baseline parameters were measured for approximately 15 min (exposure to air). The duration of exposure to PMDI was for 60 min, followed by post-challenge measurements of approximately 30 minutes. Measurements were made with eight animals simultaneously. For evaluation of responses occurring during challenge exposures the following respiratory parameters were evaluated: respiratory rate (RR) [breaths/min], tidal volume (TV) [ml], respiratory minute volume (MV) [ml/min], peak inspiratory and expiratory flow rates (PIF and PEF) [ml/sec], inspiratory (IT) and expiratory times (ET) [msec], the average duration of apnoic period (AT) [msec], and the number of apnoic periods per logging period exceeding 20% of the ET period [incidence/logging period]. Additional parameters were derived as shown in the Appendix. Measurements were made in nose-only animal restrainers with wire-mesh style pneumotachograph and differential pressure transducers (MP 45 ± 2 cm H_2O, Validyne) fitted shortly onto the plethysmograph. The head and body compartments were separated using a double-layer latex neck seal. Precautions were taken to avoid artifacts due to restraint and tight fitting seals around the neck. Volumes were calculated by integration of the flow signal from the body compartment and potential artifacts related to the dependence of the calculated volume as a function of respiratory frequency were considered. The resistance to air flow of the wire-mesh screens was adjusted so that artificial volume changes between pump rates of 50-250 cycles/min did not exceed 10%. The validation of the system was performed prior to each exposure individually for all plethysmographs using a calibration volume of 2.0 ml at a frequency of 150 cycles/min. All signals were averaged during a logging period of 20 seconds. The flow and volume signals for each individual animal were displayed on the monitor of the PC during measurement. Phase and amplitude checks were documented by re-processing of raw data.

7.14. Necropsy

Necropsy. Animals were sacrificed one day after the final challenge. Intraperitoneal injection of sodium pentobarbital (approx. 600 mg/kg b.w.) was used for euthanasia. The animals were then examined for gross pathologic changes. All findings deviating from normal were documented. Complete exsanguination was performed through cardiac puncture and the blood collected was for serological determinations.

7.15. Serological Determinations

At termination, several milliliters of blood were collected from each animal and was allowed to clot at room temperature for approximately one hour. The samples were then stored overnight at ca. 4 °C to complete the clotting process. After centrifugation, serum was collected and stored at -20 °C prior to shipping to Dr. Dearman (Zeneca CTL). Samples were sent frozen in appropriate boxes containing dry ice. Details concerning the preparation of the MDI-conjugate, its characterization, the methodology, and results of serological determinations are reported separately by Dr. Dearman (attached by the sponsor as Appendix).

7.16. Statistical Evaluation

Body weights: Body weights are tabulated in the Appendix, the mean and standard deviation (STD) is calculated. Body weight gains were analyzed by *one-way* analysis of variance and Tukey-Kramer *post hoc* test (BCTIC Computer Code Collection - Biomedical Computing Technology Information Center: ANOVA a FORTRAN Program to Perform one-way Classification Analysis of Variance. Vanderbilt Medical Center, Nashville, Tennessee, USA). The criterion for statistical significance was set at $p < 0.05$.

Lung weights: Lung weights were analyzed as absolute and relative (vs. 100 g body weight) figures. All data, including the respective body weight at sacrifice, were analyzed by *one-way* analysis of variance and Tukey-Kramer *post hoc* test (BCTIC Computer Code Collection - Biomedical Computing Technology Information Center: ANOVA a FORTRAN Program to Perform one-way Classification Analysis of Variance. Vanderbilt Medical Center, Nashville, Tennessee, USA). The criterion for statistical significance was set at $p < 0.05$.

Pulmonary function tests: Absolute and relative values for each parameter are reproduced in tabular form in the Appendix. All parameters collected are also reproduced graphically and these data were smoothed using a polynomial function before graphing (low pass filter for outliers). Brief peaks caused by abnormal movements in the plethysmograph were thereby minimized. Data in tables reflect the raw data.

One-way analysis of variances (ANOVA): In this parametric method, the data are checked for normal distribution by comparison of the median and mean values. The variances between the groups were tested for homogeneity with Box's test. If the F-test showed that the variation within the group was greater than that between the groups, this fact is indicated in the Appendix by the remark "no statistical difference between the groups". If a difference was determined, a pairwise *post-hoc* (one and two-tailed) comparison of the groups was performed using the Games and Howell modification of the *Tukey-Kramer* significance test.

Randomization: The randomization lists were produced with the aid of a computer program which used a random number generator.

Curve fitting: The analysis of linear regression curves (maximum likelihood) and iterative regression curves was made by Sigma Plot for Windows (Jandel Scientific, Erkrath, Germany).

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7.17. Reproduction of Raw Data

Raw data entered into, processed by and/or stored in a computer system could be saved and printed out in various formats. The precision (number of decimal places) of the values printed and reproduced in this report reflect toxicologically relevant levels of precision. Deviations between manually calculated and computer-determined values can arise due to rounding. Values with no decimal places do not necessarily represent the pertinent measurement precision of the detection system.

7.18. Software Programming and Validation

Software code for the following purposes was written in HP Fortran (HP 3000) or Microsoft Fortran 77 (PC): particle-size analysis, ANOVA, Fisher test, inhalation chamber data tabulation program, graphics software, physiological data evaluation. All scratch files were generated using Fortran F8.3 format using the Fortran default rounding routines. Fortran format A was always used to generate alphanumeric tables and graphs; i.e. numbers in figures and tables are rounded-up or -off due to the different format codes of the server. The computer programs were carefully validated. The validation was conducted using text book data sets (Gad and Weil, 1982). Wherever possible, raw data and calculated values are displayed graphically to provide a versatile opportunity for data comparison.

7.19. Raw Data and Report Archival

The protocol, raw data, and the final report are archived in locations specified by Bayer AG, in accordance with GLP requirements.

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8. RESULTS

Prior to the exposure to PMDI, all acutely exposed guinea pigs had been acclimatized to the restrainers in the respective nose-only inhalation chamber for 1 hour/day on three consecutive days.

8.1. Single 15-min and 60-min Exposure to PMDI

Technical details concerning the generation and characterization of the PMDI-atmosphere are summarized in Table 1. For more detailed information *cf.* Appendix.

Table 1: Generation and characterization of PMDI atmospheres - exposure: 1 x 15 min and 1 x 60 min

	Group			
Target concentration (mg/m³)	0	3	10	30
Nominal concentration (mg/m³)	-	13	44	144
Actual concentration (mg/m³)[1] - nitroreagent - HPLC analysis	-	3.7	11.2	31.4
Actual concentration (mg/m³)[1] - filter analysis	-	4.8	12.2	35.7
Flow-rate PMDI (µl/min)	0	10	10	10
Air flow - nozzle (l/min):	15	15	15	15
Dilution cascade I (l/l):	12 / 27	12 / 27	12 / 27	8.5 / 23.5
Dilution cascade II (l/l):	15 / 15	15 / 15	10 / 10	0 / 0
Dilution cascade III (l/l):	18 / 18	18 / 18	0 / 0	0 / 0
Total airflow trough chamber (l/min)	30	30	30	30
Dilution ratio:	1 : 50	1 : 50	1 : 15	1 : 1.4
Air flow - exhaust (l/min):	27	27	27	27
Temperature (°C)	21	21	21	22
Rel. humidity (%)	9	7	7	7
MMAD (µm)[1]	-	1.6	1.5	1.6
GSD	-	1.7	1.6	1.4
Aerosol Mass < 3 µm (%)	-	91	93	93
Mass recovered (mg/m³)	-	4	11	33

Dilution cascade: volume extracted / volume substituted; for calculation of the nominal concentration a density of 1 g/ml (default) was used, MMAD = Mass Median Aerodynamic Diameter; GSD = Geometric Standard Deviation, 1) The 1 x 15-min and 1 x 60-min exposures were made on the same days using the same technical set-up. Therefore the results were used for both groups. This approach is considered to be valid, since no appreciable temporal instability of test atmospheres was evident.

8.2. Single 6 hour Exposure to PMDI

Technical details concerning the generation and characterization of the PMDI-atmosphere are summarized in Table 2. For more detailed information *cf.* Appendix.

Table 2: Generation and characterization of PMDI atmospheres - exposure: 1 x 6 hr

	Group			
Target concentration (mg/m³)	0	1	3	10
Nominal concentration (mg/m³)	-	4	13	44
Actual concentration (mg/m³) - nitroreagent - HPLC analysis	-	1.4	3.0	11.9
Actual concentration (mg/m³) - filter analysis	-	1.6	3.0	12.4
Flow-rate PMDI (µl/min)	0	10	10	10
Air flow - nozzle (l/min):	30	15	15	15
Dilution cascade I (l/l):	--	12 / 27	12 / 27	8.5 / 23.5
Dilution cascade II (l/l):	--	23 / 23	15 / 15	10 / 10
Dilution cascade III (l/l):	--	22 / 22	18 / 18	0 / 0
Total airflow trough chamber (l/min):	30	30	30	30
Dilution ratio:	--	1 : 163	1 : 50	1 : 15
Air flow - exhaust (l/min):	27	27	27	27
Temperature (°C)	22	22	21	22
Rel. humidity (%)	9	7	7	7
MMAD (µm)	-	1.5	1.6	1.5
GSD	-	1.7	1.6	1.6
Aerosol Mass < 3 µm (%)	-	92	92	93
Mass recovered (mg/m³)	-	1.4	2.7	12.2

Dilution cascade: volume extracted / volume substituted; for calculation of the nominal concentration a density of 1 g/ml (default) was used

MMAD = Mass Median Aerodynamic Diameter; GSD = Geometric Standard Deviation

8.3. Repeated 6 hour Exposure to PMDI

Technical details concerning the generation and characterization of the PMDI-atmosphere are summarized in Table 3. For more detailed information *cf.* Appendix.

Table 3: Generation and characterization of PMDI atmospheres - exposure: 5 x 6 hr / week on 3 consecutive week

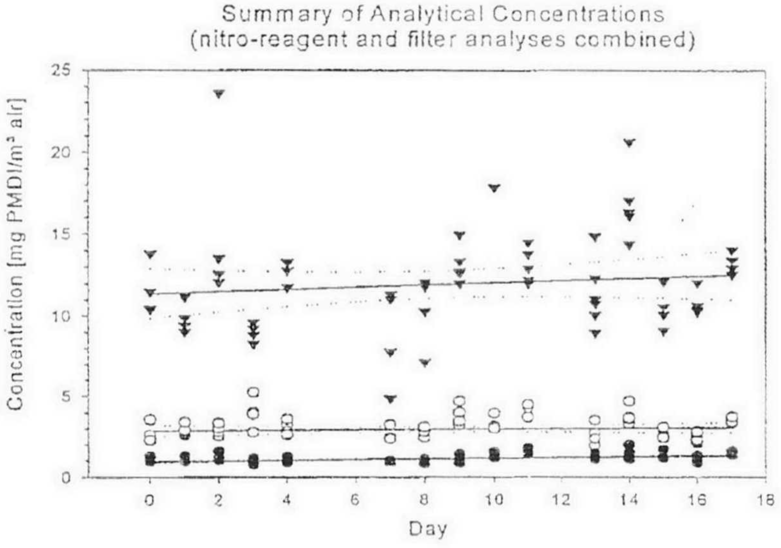
	Group			
Target concentration (mg/m³)	0	1	3	10
Nominal concentration (mg/m³)	-	3	12	33[1]
Actual concentration (mg/m³) - nitroreagent - HPLC analysis	-	1.13 ± 0.23	2.96 ± 0.57	11.94 ± 2.74
Actual concentration (mg/m³) - filter analysis	-	1.4 ± 0.26	3.7 ± 0.75	12.4 ± 0.35
Flow-rate PMDI (µl/min)	0	10	10	10
Air flow - nozzle (l/min):	15	15	15	15
Dilution cascade I (l/l):	0 / 15	13 / 28	13 / 28	13 / 28
Dilution cascade II (l/l):	---	28 / 28	22 / 22	7.5 / 7.5
Total airflow trough chamber (l/min):	30	30	30	30
Dilution ratio:	---	1 : 225	1 : 56	1 : 20
Air flow - exhaust (l/min):	27	27	27	27
Temperature (°C)	24	23	23	23
Rel. humidity (%)	2	2	1	7
MMAD (µm)	-	1.4	1.5	1.6
GSD	-	1.6	1.7	1.6
Aerosol Mass < 3 µm (%)	-	93	92	91
Mass recovered (mg/m³)	-	1.2	3.5	13.0

Dilution cascade: volume extracted / volume substituted; for calculation of the nominal concentration a density of 1 g/ml (default) was used; MMAD = Mass Median Aerodynamic Diameter; GSD = Geometric Standard Deviation

1) Based on technical settings from day 3 onwards.

The temporal stability and reproducibility of the determination of PMDI in exposure atmospheres is summarized in Fig. 4.

Figure 4: Temporal stability and reproducibility of the determination of PMDI in exposure atmospheres (dotted lines: 95% confidence intervals). From bottom to top: 1, 3 and 10 mg/m³ target groups.



Summary of generation and characterization of test atmospheres

The targeted concentrations were met and confirmed by two independent analytical methods, i.e., the isocyanate-specific nitroreagent methods and by filter analyses. The isocyanate-specific and filter-analyses were roughly identical, thus demonstrating that the isocyanate functionality of the test substance was maintained after aerosolization. Likewise, the total concentrations obtained by the critical orifice cascade impactor analyses, resulted in roughly identical concentration. This experimental evidence suggests that interstage wall-losses did not occur and that anisokinetic sampling errors were virtually negligible. Moreover, there were no appreciable concentration-dependent effects on particle-size distributions. Furthermore, the results of the particle-size analyses indicate that the aerosol was of adequate respirability and that upper respiratory tract deposition of aerosol appears to be of minor concern (Raabe *et al.*, 1988). This assumption is substantiated further by the respiratory function measurements described later. Data from individual particle analysis are reproduced in detail in the Appendix. Thus, analytical as well as real-time aerosol monitoring of each test atmosphere indicated that the exposure conditions were temporally stable during the exposure period. Indeed, as demonstrated by some examples in the Appendix, there were some shift in aerosol monitoring during the course of the 6-hour exposure periods. However, these mild shifts were apparently not confirmed by the respective analytical measurements. Therefore, these findings are considered to be associated with a deposition of PMDI-particles onto the sensing unit (photomultiplier windows) of the real-time device rather than actual shifts in atmospheric concentrations.

Temperature values were within a range recommended by the testing guidelines. Humidity values of atmospheres were, as targeted, lower than recommended. The lower humidity during exposures with aerosolized PMDI was assumed to minimize possible side reactions of the isocyanate groups with water vapor.

8.4. Toxicological Results

The results obtained during and after exposures of guinea pigs to the PMDI-aerosol atmospheres are summarized in Table 4.

Table 4: Summary of inhalation toxicity - single and repeated exposure

Exposure Regimen	Target Concentration (mg/m³)	Analytical[1] Concentration (mg/m³)	Total[1] Dose (µg/m³ x h/week)	Toxicological Result	Onset and Duration of Signs	Onset of Mortality
1 x 15 min	3	3.7	0.93	0 / 0 / 10	-	-
1 x 15 min	10	11.2	2.8	0 / 0 / 10	-	-
1 x 15 min	30	31.4	7.9	0 / 0 / 10	-	-
1 x 15 min[2]	10	11	2.8	0 / 0 / 10	-	-
1 x 15 min[2]	100	101	25.3	0 / 0 / 10	-	-
1 x 15 min[2]	900	814	203.5	0 / 0 / 10	-	-
1 x 1 hour	3	3.7	3.7	0 / 0 / 10	-	-
1 x 1 hour	10	11.2	11.2	0 / 0 / 10	-	-
1 x 1 hour	30	31.4	31.4	0 / 0 / 10	-	-
1 x 6 hours	1	1.4	8.4	0 / 0 / 10	-	-
1 x 6 hours	3	3.0	18.0	0 / 0 / 10	-	-
1 x 6 hours	10	11.9	71.4	0 / 0 / 10	-	-
3 x (5 x 6 hrs)	1	1.1	33	0 / 0 / 10	-	-
3 x (5 x 6 hrs)	3	3.0	88.8	0 / 0 / 10	-	-
3 x (5 x 6 hrs)	10	11.9	358.2	0 / 0 / 10	-	-

1) Based on nitroreagent technique, -: exposures were tolerated without any effects
2) This data stem from an earlier study (III Projects 134 & 135) and have been reported previously; Pauluhn and Dearman, 1997

Values given in the 'Toxicological results' column are:
1st = number of dead animals.
2nd = number of animals with signs after cessation of exposure
3rd = number of animals exposed.

Observations and signs:

Single exposure: The exposure was tolerated without any effect.

Repeated exposure: The exposures were tolerated without any effect.

8.5. Evaluation of sensory irritation potential

The sensory irritation potential of the PMDI-aerosol was examined on eight guinea pigs of the 1 x 1 hour exposure regimen. Results of the respiratory function measurements are also provided in the Appendix. As illustrated in Figs. 5-7, marked concentration-dependent effects on tidal volume, respiratory minute volume or respiratory rate could not be ascertained. There was, however, a mild temporal change in respiratory rate during the course of the 1-hour exposure period. Taking this into account, it appears that the guinea pigs exposed to 30 mg/m³ air experienced a PMDI-induced increase in respiratory rate. This is a common finding in rodents exposed to pulmonary irritants. The excursions observed in the range of the 15-min and 75-min time points were most likely be related to disturbances of guinea pigs as a result of connection or disconnection of the aerosol generation system.

Figure 5: Analysis of tidal volume in guinea pigs (15-min exposure to air and collection of base-line data, 1-hr exposure to the various concentrations of PMDI followed by 30-min air exposure). All data represent the means of 8 guinea pigs/group

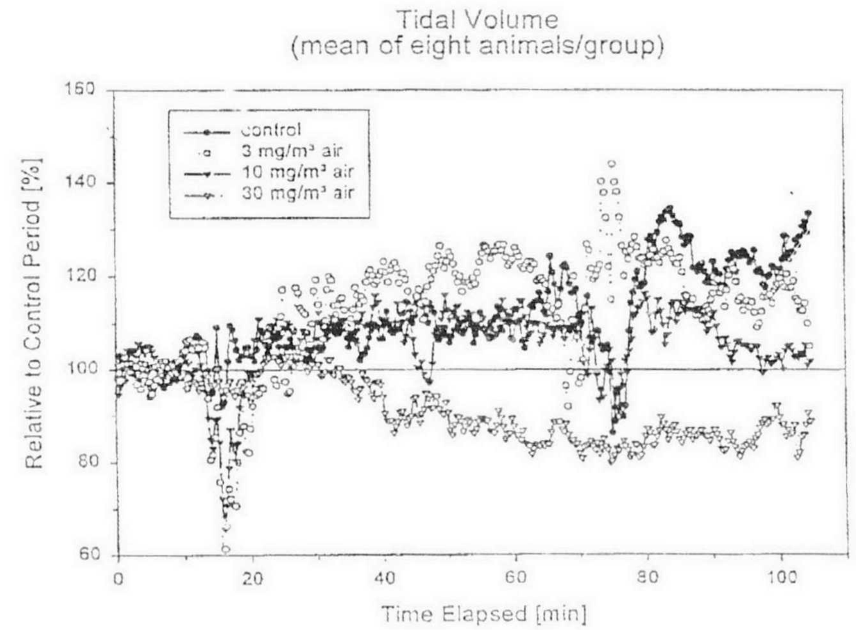


Figure 6: Analysis of respiratory minute volume in guinea pigs (15-min exposure to air and collection of base-line data, 1-hr exposure to the various concentrations of PMDI followed by 30-min air exposure). All data represent the means of 8 guinea pigs/group

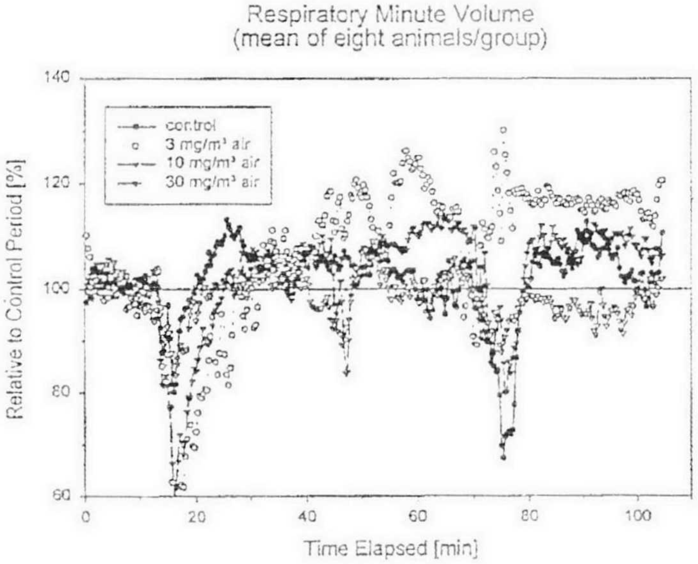
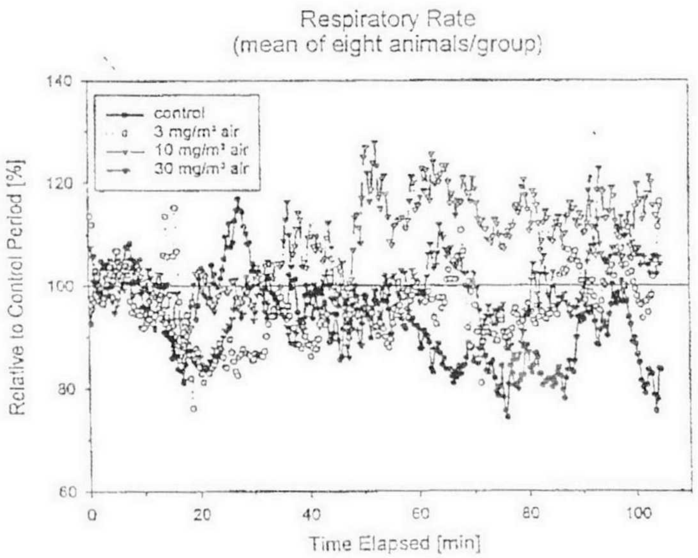


Figure 7: Analysis of respiratory rate in guinea pigs (15-min exposure to air and collection of base-line data, 1-hr exposure to the various concentrations of PMDI followed by 30-min air exposure). All data represent the means of 8 guinea pigs/group



8.6. Body weights

Results of the statistical evaluation of the body weights are included in the Appendix, mean body weights are depicted in Figs. 8 (1 x 15-min), 9 (1 x 60-min), 10 (1 x 6 hr), and 11 (5 x 6 hr/week on 3 consecutive weeks).

In the acutely exposed animals, comparisons between control animals with those in the various exposure groups revealed some mild and inconsistent effects on body weights which are not considered related to PMDI exposure. Following repeated exposure, in the last week a mild decrease in body weight gain was observed in the 10 mg/m³ air group, however, this effect did not gain statistical significance (see Appendix pp. 130 - 138).

Figure 8: Body Weights (means ± standard deviation)

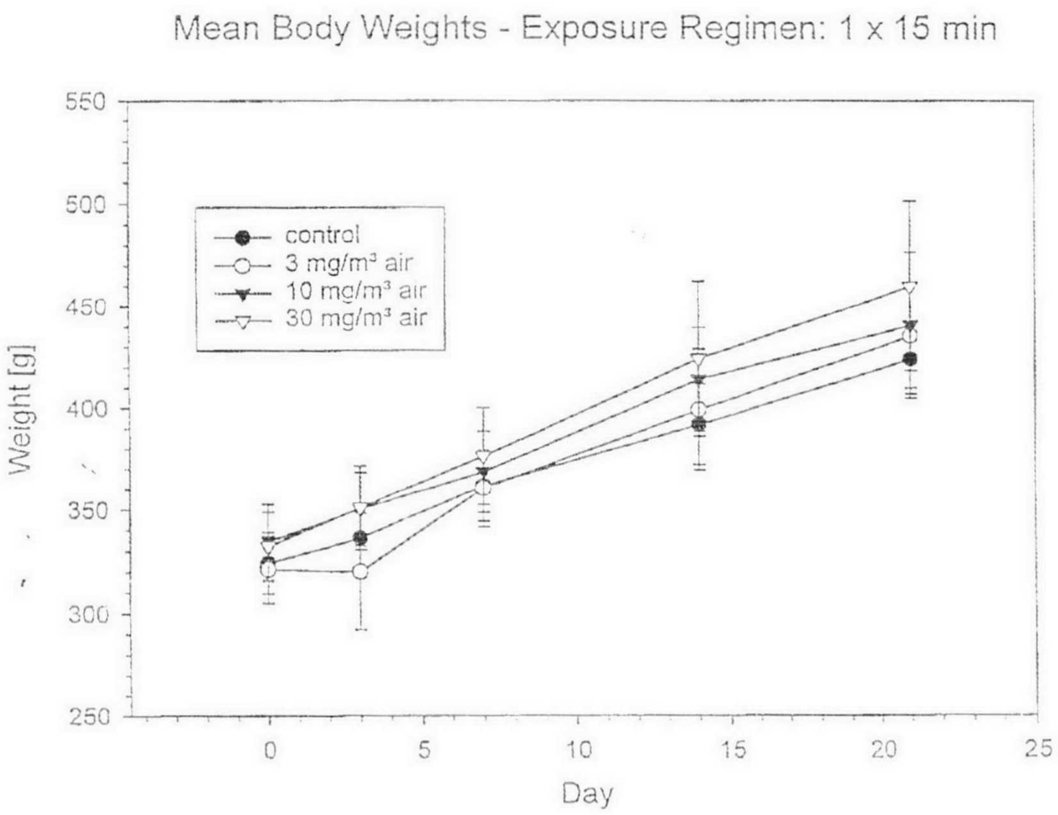


Figure 9: Body Weights (means ± standard deviation)

Mean Body Weights - Exposure Regimen: 1 x 1 hr

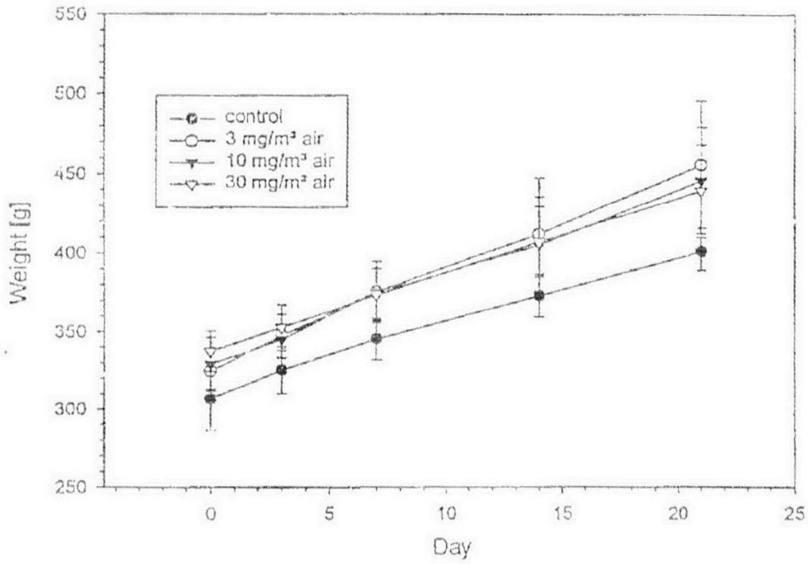


Figure 10: Body Weights (means ± standard deviation)

Mean Body Weights - Exposure Regimen: 1 x [illegible] hr

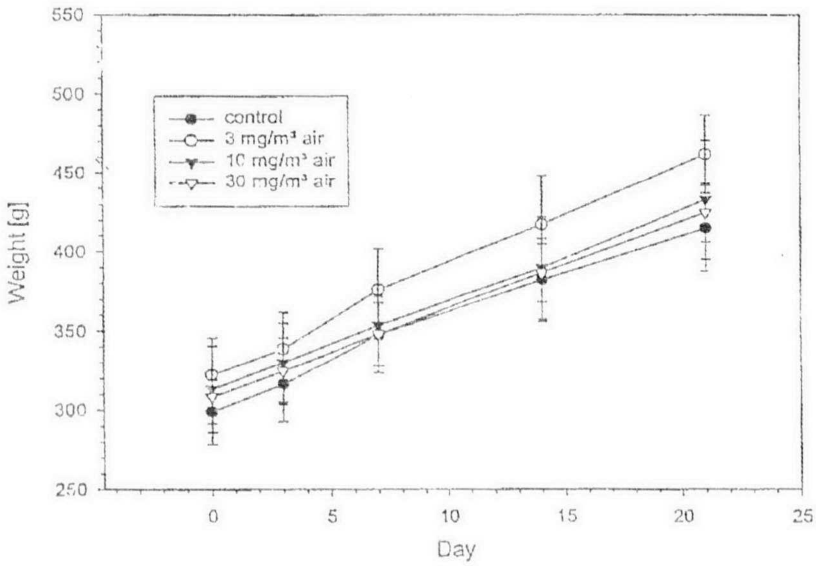
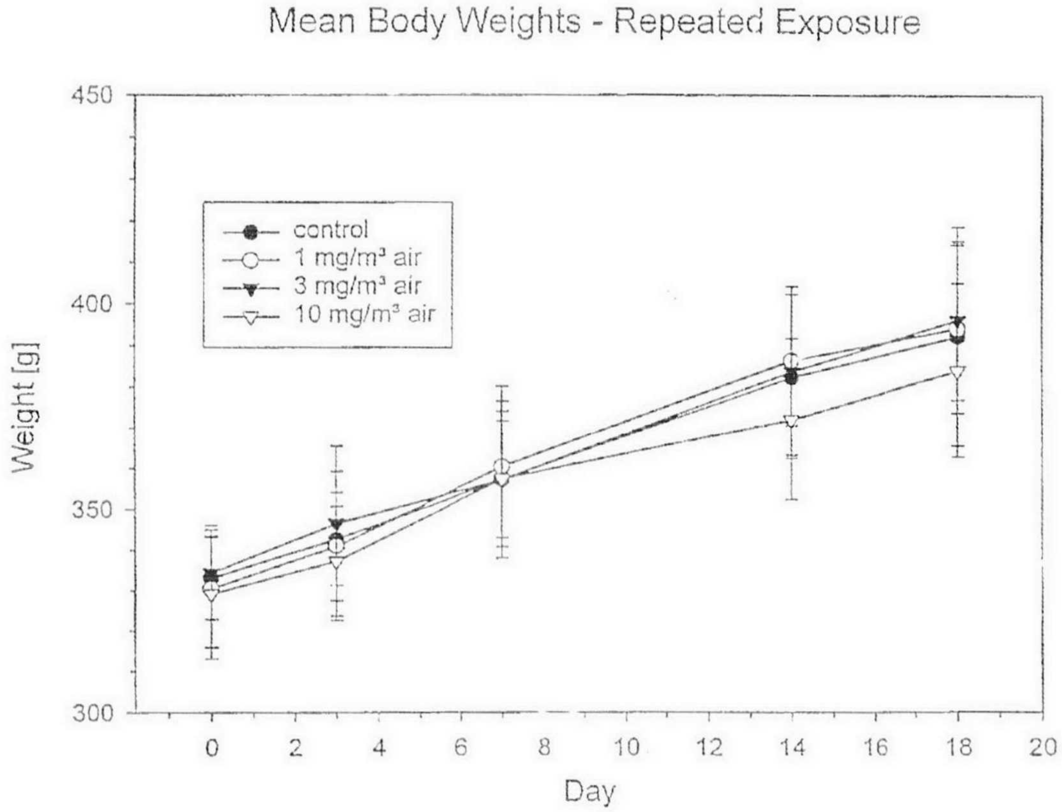


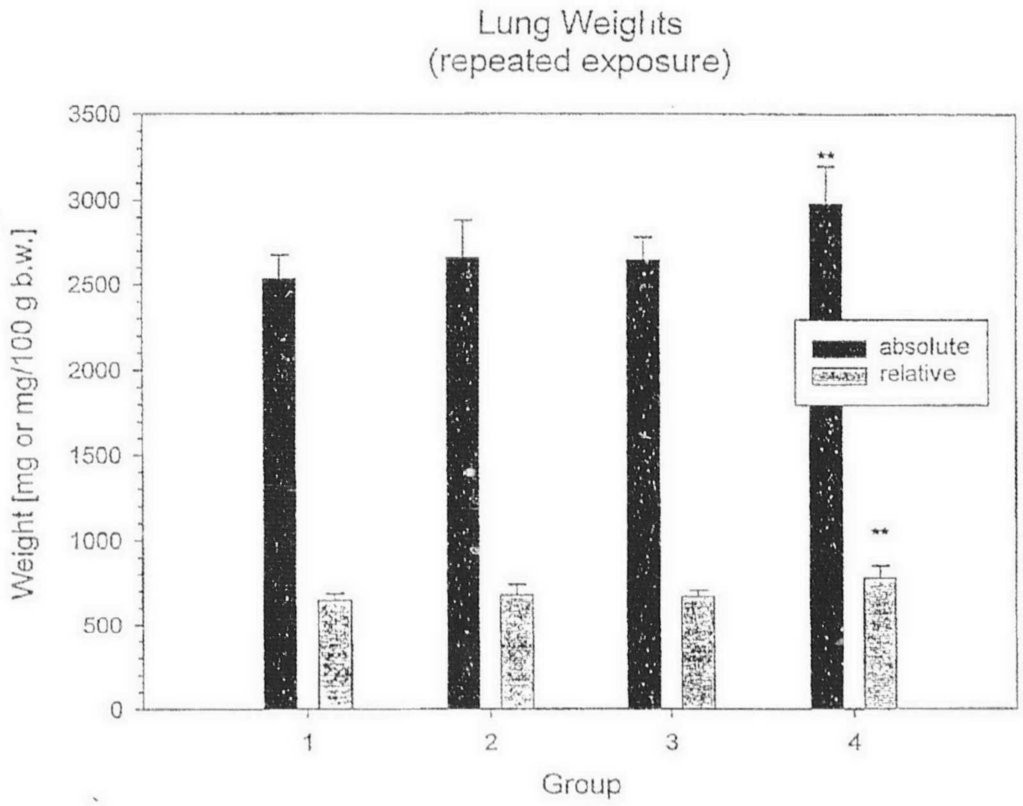
Figure 11: Body Weights (means ± standard deviation)



8.7. Lung weights

Results of the statistical evaluation of the lung weights are included in the Appendix. Guinea pigs acutely exposed to PMDI and sacrificed 3 weeks after exposure did not show any conclusive effects on lung weights. Following repeated exposure to PMDI, the lung weights of the 10 mg/m³ group were statistically significantly increased. The overall effect of mean absolute and relative lung weights are depicted in Fig. 12.

Figure 12: Absolute and relative lung weights (means ± standard deviation) of guinea pigs exposed to PMDI 5 x 6 hr/day on 3 consecutive weeks. Group 1: concurrent air control, group 2: 1 mg/m³ air, group 3: 3 mg/m³ air, group 4: 10 mg/m³ air (for individual data see Appendix pp. 139 - 143).



8.8. Necropsy

A detailed listing of the individual findings is included in the Appendix. An incidence table addressing the macroscopic lung findings is given in the Appendix pp. 146.

The gross pathological examinations in actually exposed guinea pigs showed no PMDI induced changes. In guinea pigs of the repeated exposure regimen, there was an increased incidence of specific findings in the 10 mg/m³ exposure group. These changes included: a distended lung following opening of the thoracic cavity, dark-red discolorations and consolidation of lungs, enlarged lung-associated lymph-nodes, and an apparent congestion of ventricular vessels of the heart.

8.9. Serology

The results of the IgG_1-anti MDI-antibody determinations demonstrated anti-MDI antibody titers in the majority of animals sensitized to PMDI. Details are reported separately.

The results of current and previous (Pauluhn and Dearman, 1997) determinations are summarized in Figs. 13 and 14. Attempts were made to correlate current and previous IgG1-anti MDI-antibody determinations with the exposure regimens and cumulative PMDI dose (see Table 4 and Discussion and Assessment).

Figure 13: Individual data of IgG1-anti MDI-antibody determinations.

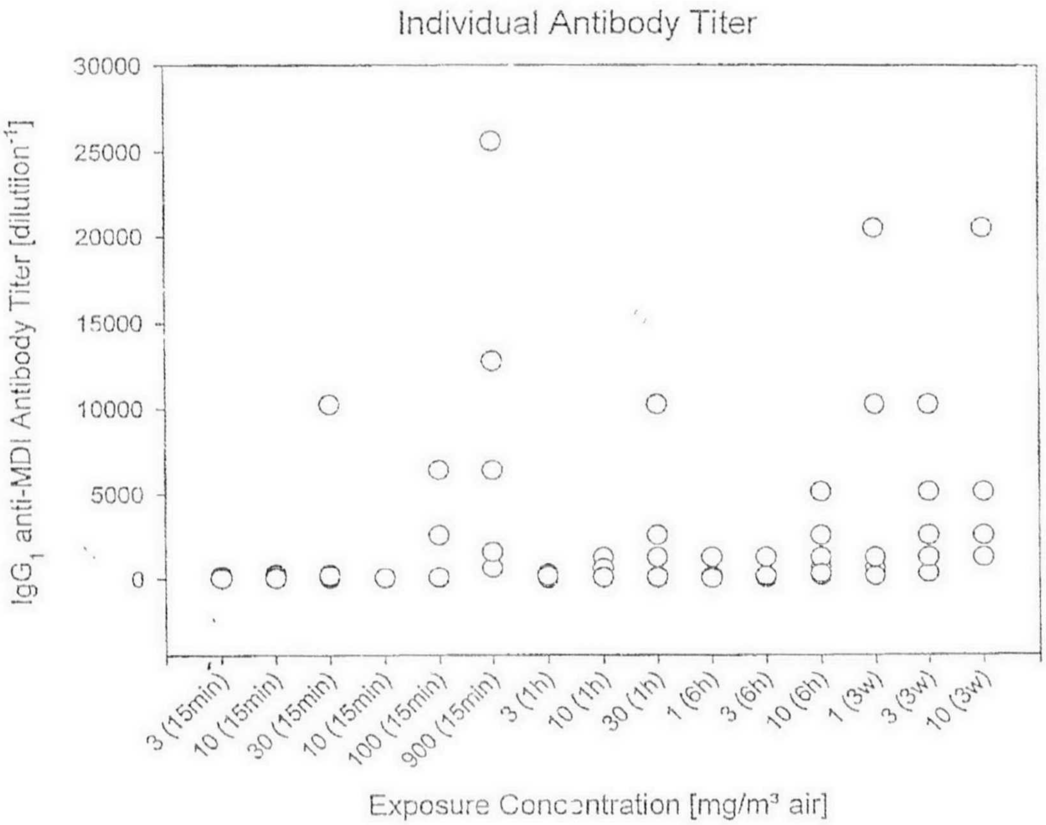
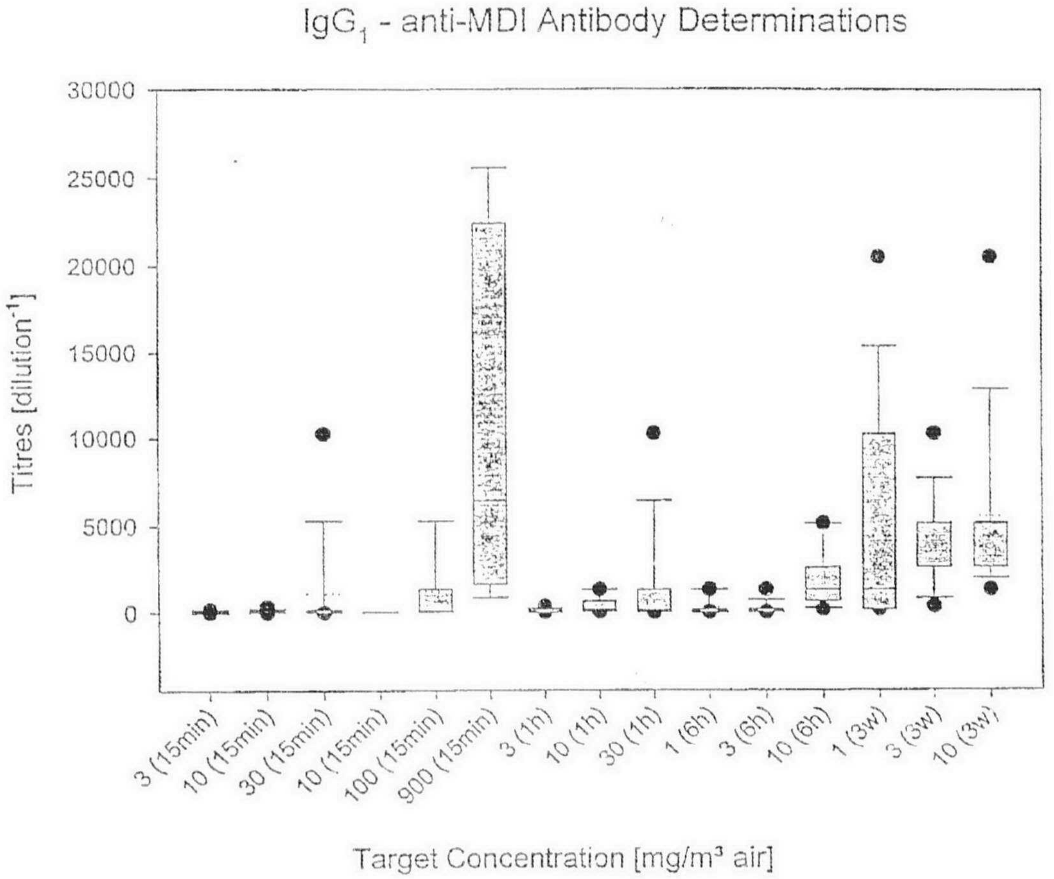


Figure 14: Box-plot of IgG_1-anti MDI-antibody determinations.



Legend: This box plot type of graph displays the 10th and 90th percentiles as bottom and top lines on a bar centered about the median (solid line) and mean (dotted line), and the 5th and 95th percentiles as whiskers. The data points beyond the 5th and 95th percentiles are also shown.

9. DISCUSSION AND ASSESSMENT

A lung sensitization study with *polymeric* MDI (PMDI) was made using guinea pigs of the Hartley strain. This approach used either a single or repeated 3-week inhalation exposure regimen for the sensitization of animals. Attempts were made to make dosimetric adjustments for exposure concentration x time relationships and IgG_1-anti MDI-antibody levels. No attempt was made to calculate the actually inhaled dose.

Following single and repeated inhalation induction, PMDI-exposed animals did not display any difference in clinical appearance when compared with the respective air control group. Body weight gains were not markedly different from the concurrent control groups. Repeatedly exposed animals of the 10 mg/m³ 3-week exposure group, however, showed a mild reduction in body weight gain towards the end of study. In this group the absolute and relative lung weights were statistically significantly increased whilst in the remaining groups the lung weights were indistinguishable from the concurrent control group.

The animals in the various single-exposure PMDI-induction groups displayed a concentration x time-dependent IgG_1-anti MDI-antibody response (Figs. 15 - 17). In animals of the 3-week inhalation regimen such relationship could not be established. In none of the control animals IgG_1-anti MDI-antibody were detectable. The lack of a concentration-dependent IgG_1-anti MDI-antibody response in the repeated exposure inhalation study remains puzzling, since in the 10 mg/m³ exposure group the prevailing experimental findings suggest PMDI-induced lung irritation whilst the 1 and 3 mg/m³ groups appeared to be indistinguishable from controls. As illustrated by the analytical characterization of test atmospheres as well as by the continuous real-time monitoring of atmospheres, there were no apparent short-term peak excursions in exposure concentrations in the 1 and 3 mg/m³ groups. Therefore, this finding suggests a total-dose rather than a concentration-dependent phenomenon.

Taking into account the intensity and duration of exposure, serological data show some concentration x time relationships. It appears, however, that high concentrations during a short period of time are more critical for IgG_1-anti MDI-antibody induction than lower concentrations during a longer period of time (Fig. 17). This means, despite increased cumulative dose, there is a lack of a proportional increase in antibody production. The comparison of exposure concentrations with the respective cumulative concentration x time relationships appears to suggest that IgG_1-anti MDI-antibody production is a saturable process and that for the repeated exposure regimen the maximum response was apparently attained in all MDI-exposure groups. However, one major difference of the single and repeated exposure regimens is that the animals were sacrificed after a 3-week postexposure period and 1-day after the last exposure, respectively.

4

Figure 15: Association of IgG_1-anti MDI-antibody determinations vs. exposure concentration and duration of exposure

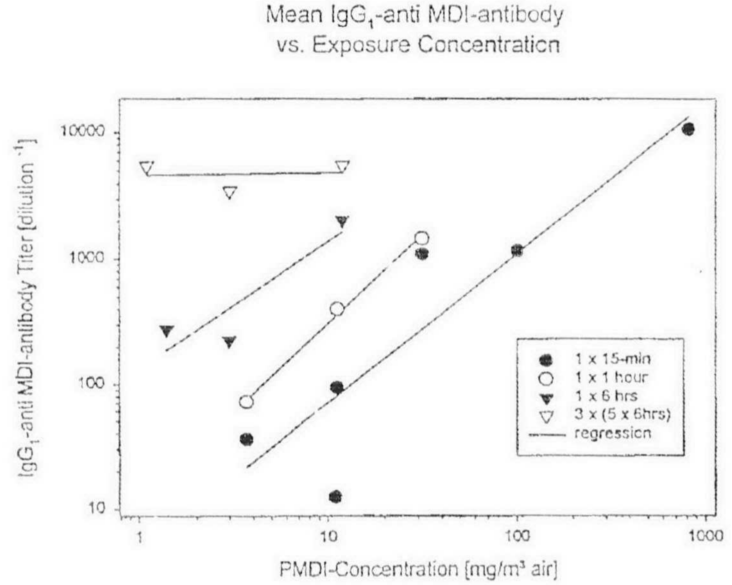


Figure 16: Association of IgG_1-anti MDI-antibody determinations vs. exposure dose (concentration x time)

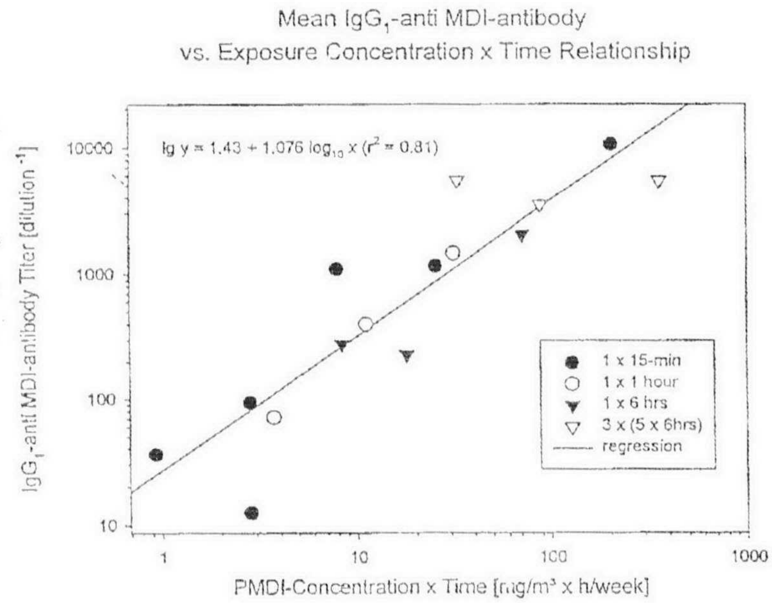
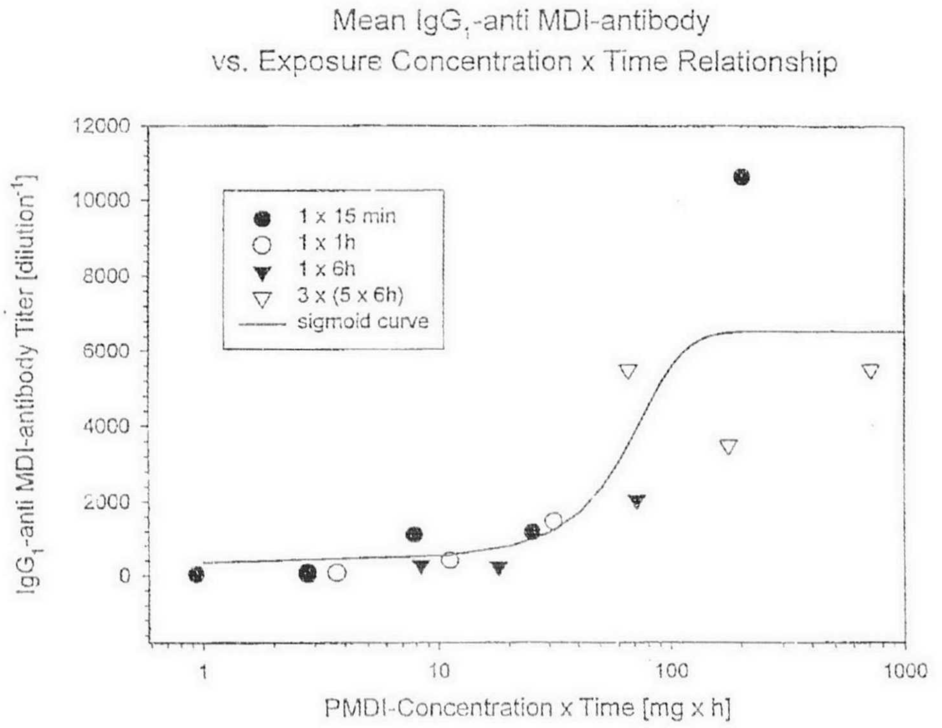


Figure 17: Association of IgG_1-anti MDI-antibody determinations vs. exposure dose (concentration x time) - Analysis of saturation of response



y = 10 + a/(1 + exp(-(x-x0)/b))
a = 6512
b = 21.3
x0 = 63

This type of saturation of the IgG_1-anti MDI-antibody response has already been described in context with toluene diisocyanate (TDI) using a 5 days, 3-hr/day (cumulative exposure duration: 15-hr) regimen (Karol, 1983). Saturation appears to occur at TDI exposure levels equal to or exceeding ≈ 4 mg/m³ air. Assuming equal potency and taking into account that in this study the cumulative exposure duration was 90-hr, saturation of the IgG_1-anti MDI-antibody response should occur below 1 mg PMDI/m³ air. The principles of IgG_1-antibody response, increased pulmonary responsiveness and airway eosinophilia and their relevance to humans has been discussed in detail elsewhere (Karol, 1983; Karol et al., 1997)

If one would consider the repeated exposure to be also a possible re-challenge type of exposure, then IgG_1-anti MDI-antibodies may have been sequestered at the location of first contact with the inciting agent, *viz.*, the respiratory tract. Therefore, due to the absence of any re-challenge type of exposure, the results obtained by single and repeated exposures cannot directly be compared, since antibody levels in the peripheral blood may not necessarily reflect those of the lung or are affected in a manner difficult to quantify.

In summary, following dosimetric adjustment, a concentration x time relationship appears to exist when the results of all studies are summarized (Fig. 16 and 17). Taking into account the linear relationship depicted in Fig. 16, the linear regression of antibody titers vs. concentration x time, shows the following relationship: $\log_{10}$ y [IgG_1-anti MDI-antibody titer] = 1.43 + 1.076 $\log_{10}$ (c x t) [concentration x h/week]. Also the sigmoid analysis suggests that antibody-production is a saturable process, however, the intensity of response appears to demonstrate strong dependence on the protocol used for induction, i.e., whether a single high-level or repeated low-level exposure regimen was used.

10. KEY TO ABBREVIATIONS

MMAD	Mass Median Aerodynamic Diameter
NMAD	Number Median Aerodynamic Diameter
GSD	Geometric standard deviation (GSD)
ECD	Effective cut-off diameter
Ai	Sample for analysis
A.U.	Arbitrary Units
STAND, S, Std, s	Standard deviation
MW/MEANS	Means
F	F-test value (F-ratio)
DF	Degrees of freedom
PROB	Probability
SS	Total sum of squares
MS	Mean squares
TREATMENT	- between the groups
ERROR	- within the groups
TOTAL	- total

<u>ORGAN WEIGHTS</u>

absolute -	all data in mg
relative vs. body weight (b.w.)-	all data in mg/100 g b.w.

<u>STATISTICS</u>

STAND, S, Std	standard deviation
MW / MEANS, $\bar{x}$	means
+/*	Difference against control for $p \leq 0.05$ significant
++/**	Difference against control for $p \leq 0.01$ significant
F	F-test-value (F-Ratio)
DF	degrees of freedom
PROB	probability
SS	Total sum of squares
MS	Mean squares
TREATMENT	- between the groups
ERROR	- within the groups
TOTAL	- total

11. REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists) (1978) Air Sampling Instruments for Evaluation of Atmospheric Contaminants, 5th Edition, ACGIH p. F-6. ACGIH section I: Calibration of Air Sampling Instruments and section F: Aerosol Sampling for Particle Size Analysis.

CHEMG (1994). Principles of Good Laboratory Practice, BGBl [federal law gazette], dated July 29, 1994.

Dennis R.(1976). Handbook of Aerosols - Technical Information Center, Energy Research and Development Administration, S. 110-114, July 1976.

EG Guideline 86/609/EC (1986). Guideline of the Council dated November 24, 1986 on the Reconciliation of Legal and Administrative Regulations of the Member Countries for the Protection of Animals used for Studies and other Scientific Purposes. Journal of the European Community, Legal Specifications L 358, 29.

EG Guideline 84/449 (1984). Journal of the European Community - Legal Specifications L 251, 27, September 19, 1984. B.2. Acute Toxicity - Inhalation. p. 99

Gad SC, Weil CS (1982). Statistics for Toxicologists. Principles and Methods of Toxicology, ed. A.W. Hayes, Raven Press, New York, p. 280.

Greenspan L (1977). Humidity Fixed Points of Binary Saturated Aqueous Solutions, Journal of Research of the National Bureau of Standards, Vol. 81 A, no. 1, Jan.-Febr. 1977.

Karol M.H. (1983). Concentration-dependent immunologic response to toluene diisocyanate (TDI) following inhalation exposure. *Toxicol. Appl. Pharmacol.* **68**, 229-241.

Karol M.H., Jin R. Lantz R.C. (1997). Immunohistochemical detection of toluene diisocyanate (TDI) adducts in pulmonary tissue of guinea pigs following inhalation exposure. Inhalation Toxicology 9:63-83.

Lushniak B.D., Reh C.M., Bernstein D.I., and Gallagher J.S. (1998). Indirect Assessment of 4,4'-Diphenylmethane Diisocyanate (MDI) Exposure by Evaluation of Specific Humoral Immune Responses to MDI Conjugated to Human Serum Albumin. Am. J. Ind. Med. **33**: 471 - 477.

Marple VA and Rubow, KL (1980). Aerosol Generation Concepts and Parameters in Generation of Aerosols and Facilities for Exposure Experiments, Ed. K. Willeke, Ann Arbor Science Publ. Inc. Mich., pp. 3-29.

McFarland HN (1976). Respiratory Toxicology - Essays in Toxicology, Vol. 7, pp. 121-154, Academic Press Inc., New York, San Francisco, London.

Moss and Asgharian B (1994). Precise inhalation dosimetry with minimum consumption of product: The challenge of operating inhalation exposure systems at ther design limits. Respiratory Drug Delivery IV pp. 197-201.

OECD - GLP (1983). Publication of the German version of the OECD Principles of Good Laboratory Practice (GLP), *Bundesanzeiger*, 35, No. 42a dated March 2, 1983.

OECD-Guideline for Testing of Chemicals No. 403. "Acute Inhalation Toxicity", adopted May 12 (1983).

Pauluhn J (1988). Different methods used in acute and subchronic inhalation studies of potential lung irritants, with particular attention to lung function measurements. In U. Mohr, ed., Inhalation

Al

Toxicology, The design and interpretation of inhalation studies and their use in risk assessment. pp. 87-101. Springer Verlag Heidelberg.

Pauluhn J (1986). Study to Determine Temperature and Humidity Data in Inhalation Chambers; BAYER AG Report No. 15007 dated August 22.

Pauluhn J, Eben A. (1991). Validation of a non-invasive technique to assess immediate or delayed onset of airway hypersensitivity in guinea-pigs. J. Appl. Toxicol. 11: 423-431.

Pauluhn J (1994). Validation of an improved nose-only exposure system for rodents. Journal of Applied Toxicology, 14:55-62.

Pauluhn J and Dearmar [illegible]997). Polymeric MDI: Evaluation of respiratory hyperreactivity in rats and induction of Ig[illegible] anti MDI-antibodies in guinea pigs following brief, high-level inhalation induction exposure. III Projects 134 & 135, III ref. 11269.

Raabe OG (1982). Deposition and Clearance of Inhaled Aerosols in H. Witschi and P. Nettesheim - Mechanisms in Respiratory Toxicology Vol. I, pp. 27-76, CRC Press, Inc. Boca Raton, Florida.

Raabe OG, Al-Bayati MA, Teague SV and Rasolt A (1988). Regional deposition of inhaled monodisperse coarse and fine aerosol particles in small laboratory animals. Ann occup. Hyg. 32:53-63.

Snipes MB (1989). Long-Term Retention and Clearance of Particles Inhaled by Mammalian Species. Critical Reviews in Toxicology, Vol. 20, pp. 175-211.

SOT-COMMENTARY (1992). Recommendations for the Conduct of Acute Inhalation Limit Tests, prepared by the Technical Committee of the Inhalation Speciality Section, Society of Toxicology. Fundam. Appl. Toxicol. 18, pp. 321-327.

Tillery MI, Wood GO and Ettinger JJ (1976). Generation and Characterization of Aerosols and Vapors for Inhalation Experiments. Environmental Health Perspectives 16, pp. 25-40.

U.S. Environmental Protection Agency (1988). Hazard evaluation division: Standard evaluation procedure, inhalation toxicity testing, NTIS Report PB89-100366, Washington, DC.

D 13

III Project 153

III ref. 11322 Volume 2

Diphenylmethane 4,4'-diisocyanate (MDI-polymer) Evaluation of respiratory sensitisation in guinea-pigs following single high-level and repeated low-level inhalation induction exposure

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Germany

Number of pages: 123

12. APPENDIX - SINGLE EXPOSURE

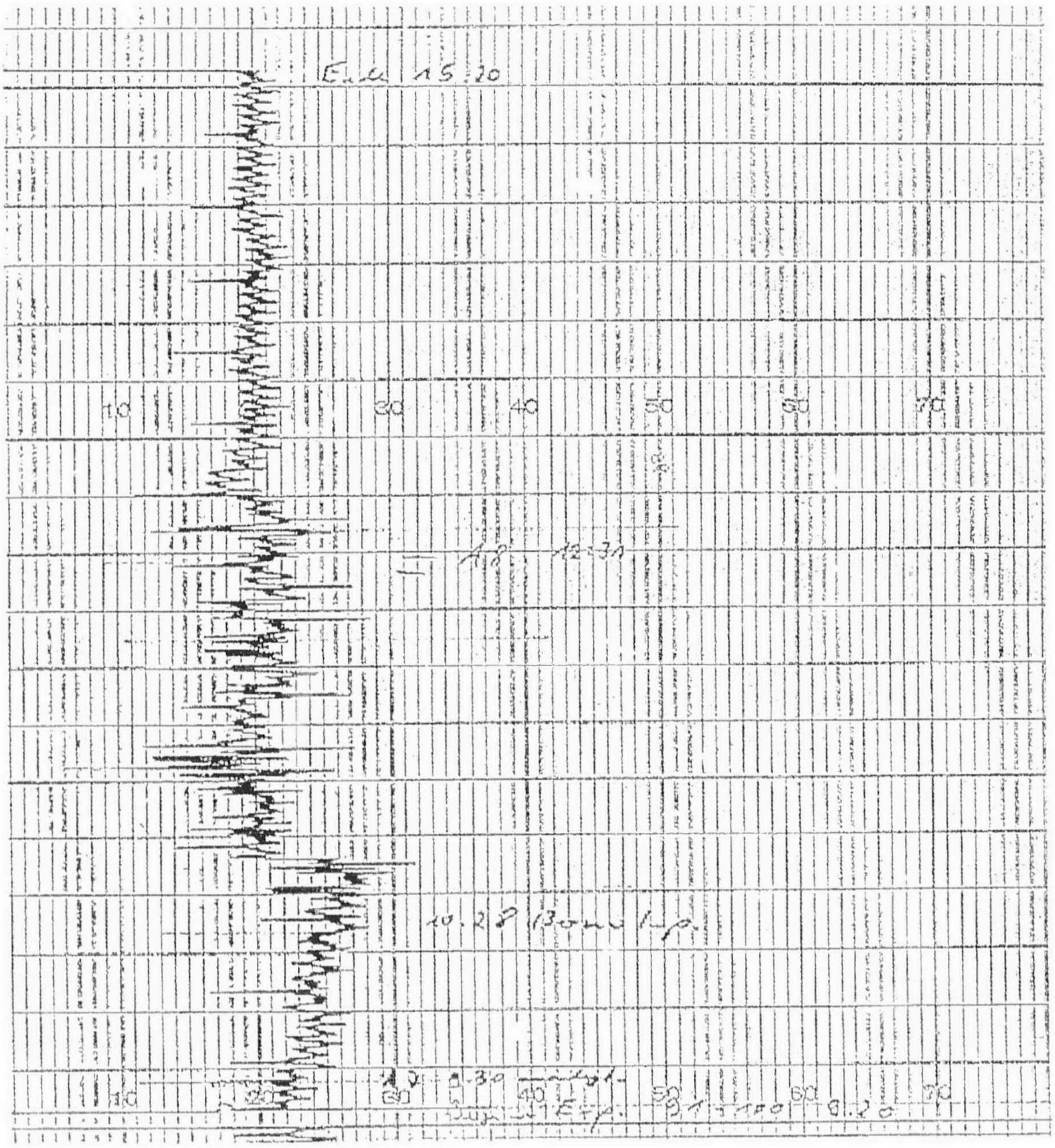
Exposure Regimen and Atmosphere Characterization

Group	Date of exposure (DD.MM.YY)	Animal-no.	Exposure Regimen	Target Conc (mg/m³ air)	Concen-tration (mg/m³ air) **Nitroreagent**	Concen-tration (mg/m³ air) **Filter**
1	26.01.1998	1-10	1 x 15 min	0	--	--
2	26.01.1998	11-20	1 x 15 min	3	3.7	4.8
3	28.01.1998	21-30	1 x 15 min	10	11.2	12.2
4	28.01.1998	31-40	1 x 15 min	30	31.4	35.7
5	26.01.1998	41-50	1 x 1 hr	0	--	--
6	26.01.1998	51-60	1 x 1 hr	3	3.7	4.8
7	28.01.1998	61-70	1 x 1 hr	10	11.2	12.2
8	28.01.1998	71-80	1 x 1 hr	30	31.4	35.7
9	26.01.1998	81-90	1 x 6 hr	0	--	--
10	26.01.1998	91-100	1 x 6 hr	1	1.4	1.6
11	28.01.1998	101-110	1 x 6 hr	3	3.0	3.0
12	28.01.1998	111-120	1 x 6 hr	10	11.9	12.4

In the subsequently presented tables target concentrations are used to indicate the respective group.

Monitoring of Atmosphere (Examples)

1 x 6 hr - 1 mg/m³ air

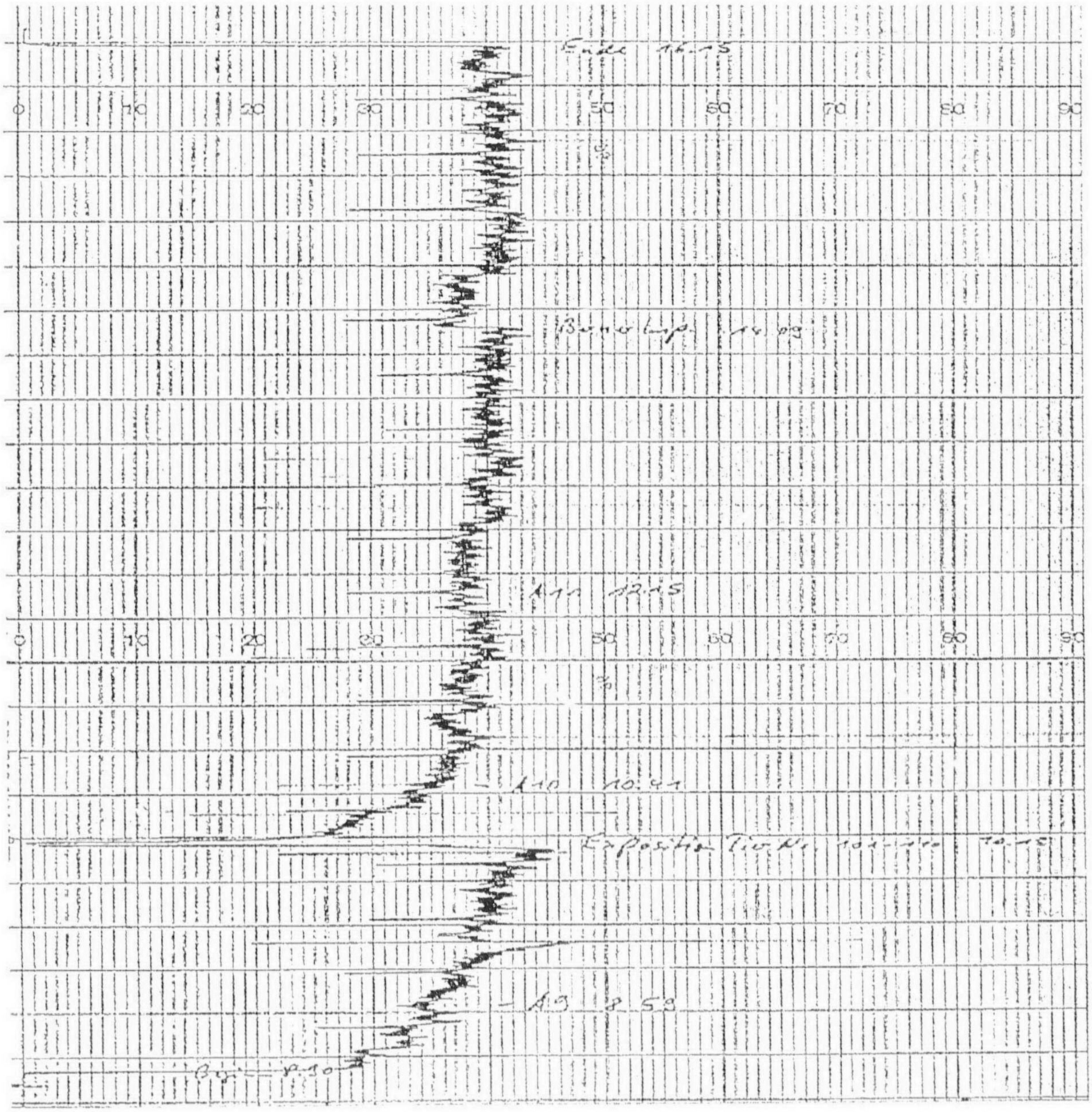


Legend (copy of raw data):

Ai: i-th analytical sample, Berner-Imp.: cascade impactor sampling
Beginn Exp.: start of exposure, Ende: End, time: mm.hh

Monitoring of Atmosphere (Examples)

1 x 6 hr - 3 mg/m³ air



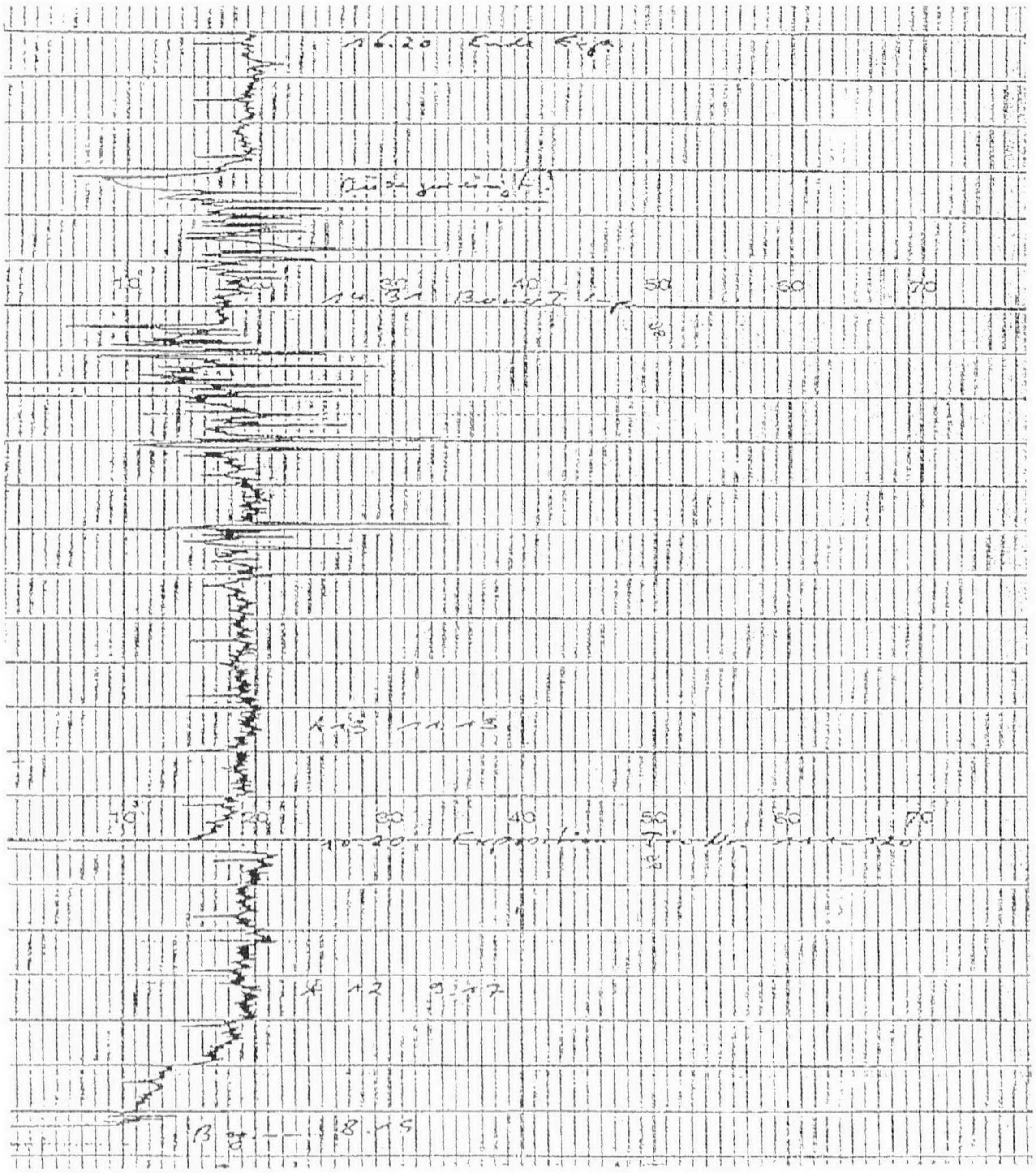
Legend (copy of raw data):

Ai: i-th analytical sample, Berner-Imp.: cascade impactor sampling
Beginn Exp.: start of exposure, Ende: End, time: mm.hh

E 03

Monitoring of Atmosphere (Examples)

1 x 6 hr - 10 mg/m³ air



Legend (copy of raw data):

Ai: i-th analytical sample, Berner-Imp.: cascade impactor sampling
Beginn Exp.: start of exposure, Ende: End, time: mm.hh

E 04

Particle-size Characterization of Test Atmosphere

Group	Date of exposure (DD.MM.YY)	Target Conc. (mg/m³ air)	MMAD [µm]	GSD	Mass ≤ 3 µm [%]	Conc. (mg/m³ air) Impactor
1	26.01.1998	0	--	--	--	--
2	26.01.1998	3	--	--	--	--
3	28.01.1998	10	--	--	--	--
4	28.01.1998	30	--	--	--	--
5	26.01.1998	0	--	--	--	--
6*	26.01.1998	3	1.55	1.65	91	4.0
7*	28.01.1998	10	1.53	1.60	93	11.1
8*	28.01.1998	30	1.61	1.52	93	32.8
9	26.01.1998	0	--	--	--	--
10	26.01.1998	1	1.45	1.67	92	1.4
11	28.01.1998	3	1.56	1.61	92	2.7
12	28.01.1998	10	1.54	1.57	93	12.2

--: due to the short duration of exposure no particle-size analysis performed
*) Examples of these evaluation of particle-size distributions are provided on the next pages.

Characterization of Particle Size Distribution (Examples)

ANALYSIS OF PARTICLE DISTRIBUTIONS

Type of investigation: Acute Inhalation - Aerosol

Compound: PMDI

Date of exposure: 02.02.1998 Study-no.: T7062289
Nominal concentration: 1.0 mg/m3 air

N	Impactor stage (um - um)	Cut-Off diameter (um)	Mass/ stage (mg)	Rel. mass (%)	Cumul. mass (%)
1	.06 - .12	.060	.003	.31	.00
2	.12 - .25	.120	.006	.62	.31
3	.25 - .49	.250	.014	1.45	.93
4	.49 - .90	.490	.115	11.94	2.39
5	.90 - 1.85	.900	.507	52.65	14.33
6	1.85 - 3.69	1.850	.287	29.80	66.98
7	3.69 - 7.42	3.690	.027	2.80	96.78
8	7.42 -14.80	7.420	.004	.42	99.58
9	14.80 -30.00	14.800	.000	.00	100.00

Mass Median Aerodynamic Diameter (MMAD): 1.45 um
Geometric standard deviation (GSD): 1.68
Number Median Aerodynamic Diameter (NMAD): .65 um
Surface Median Aerodynamic Diameter (SMAD): 1.11 um

System: BERNER-IMPACTOR I
Air flow: 5.66 liter/min.
Sampling time: 7200.00 seconds
Concentration (computed): 1.42 mg/m3 air

Respirability (percent < 1.0 um):

1. Mass related: 23.7 % (measured)
2. Number related: 79.7 % (extrapolated)

Respirability (percent < 3.0 um):

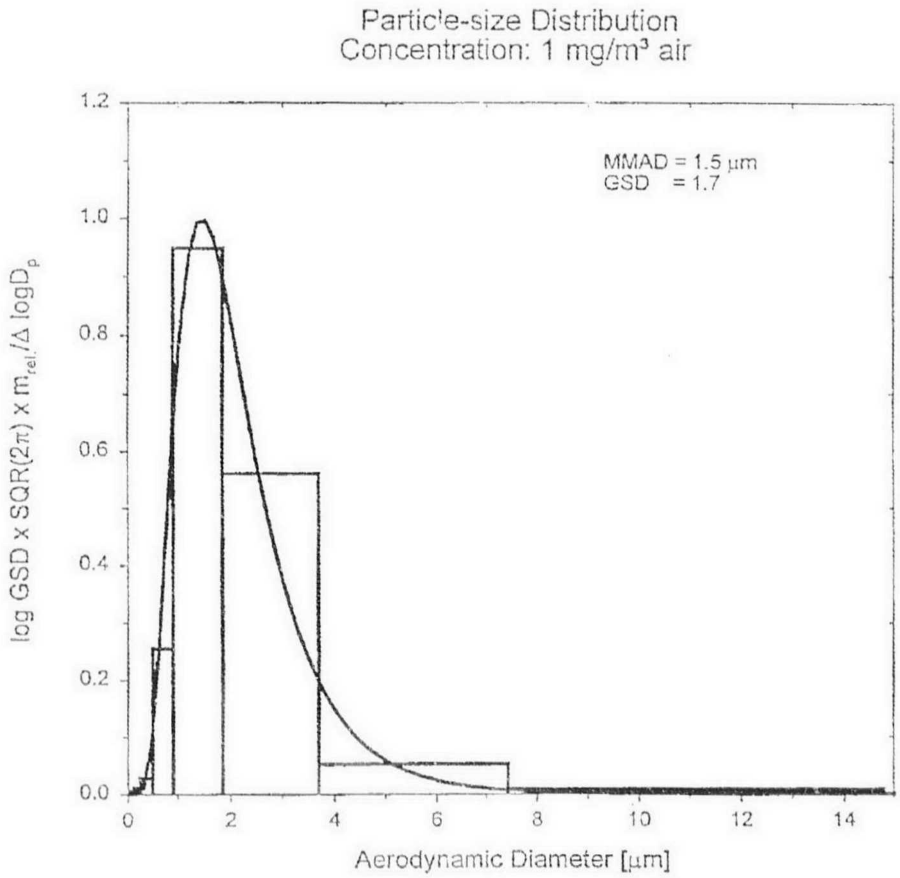
1. Mass related: 91.9 % (measured)
2. Number related: 99.1 % (extrapolated)

Respirability (percent < 5.0 um):

1. Mass related: 99.1 % (measured)
2. Number related: 99.1 % (extrapolated)

ECD-definition: right cut-size (Dp+1)

E 06


Particle-size Distribution
Concentration: 1 mg/m³ air
MMAD = 1.5 µm
GSD = 1.7
log GSD x SQR(2π) x m_rel/Δ logD_p
Aerodynamic Diameter [µm]
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0
2
4
6
8
10
12
14

ANALYSIS OF PARTICLE DISTRIBUTIONS

Type of investigation: Acute Inhalation - Aerosol

Compound: PMDI

Date of exposure: 26.01.1998 Study-no.: T7062289
Nominal concentration: 3.0 mg/m³ air

N	Impactor stage (um - um)	Cut-Off diameter (um)	Mass/ stage (mg)	Rel. mass (%)	Cumul. mass (%)
1	.06 - .12	.060	.004	.30	.00
2	.12 - .25	.120	.007	.52	.30
3	.25 - .49	.250	.012	.89	.81
4	.49 - .90	.490	.107	7.91	1.70
5	.90 - 1.85	.900	.738	54.55	9.61
6	1.85 - 3.69	1.850	.431	31.86	64.15
7	3.69 - 7.42	3.690	.047	3.47	96.01
8	7.42 -14.80	7.420	.007	.52	99.48
9	14.80 -30.00	14.800	.000	.00	100.00

Mass Median Aerodynamic Diameter (MMAD): 1.56 um
Geometric standard deviation (GSD): 1.66
Number Median Aerodynamic Diameter (NMAD): .72 um
Surface Median Aerodynamic Diameter (SMAD): 1.20 um

System: BERNER-IMPACTOR I
Air flow: 5.66 liter/min.
Sampling time: 3600.00 seconds
Concentration (computed): 3.98 mg/m³ air

Respirability (percent < 1.0 um):

1. Mass related: 19.3 % (measured)
2. Number related: 73.9 % (extrapolated)

Respirability (percent < 3.0 um):

1. Mass related: 90.3 % (measured)
2. Number related: 99.1 % (extrapolated)

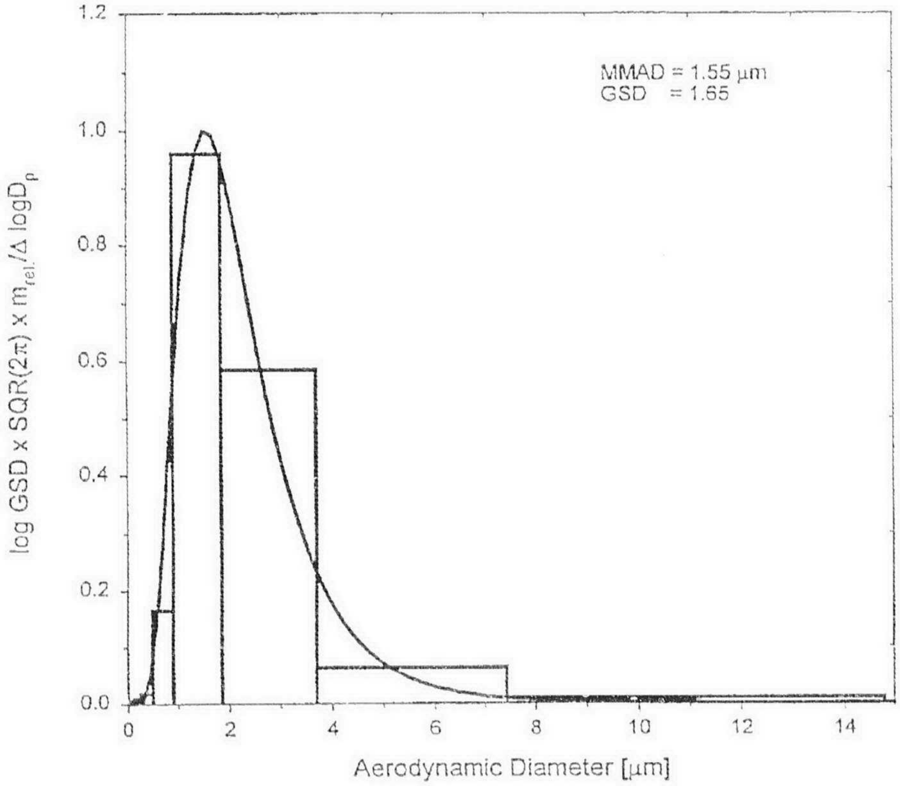
Respirability (percent < 5.0 um):

1. Mass related: 99.1 % (measured)
2. Number related: 99.1 % (extrapolated)

ECD-definition: right cut-size (Dp+1)

u

Particle-size Distribution
Concentration: 3 mg/m³ air



ANALYSIS OF PARTICLE DISTRIBUTIONS

Type of investigation: Acute Inhalation - Aerosol

Compound: PMDI

Date of exposure: 28.01.1998 Study-no.: T7062289
Nominal concentration: 10.0 mg/m³ air

N	Impactor stage (um - um)	Cut-Off diameter (um)	Mass/ stage (mg)	Rel. mass (%)	Cumul. mass (%)
1	.06 - .12	.060	.001	.06	.00
2	.12 - .25	.120	.007	.44	.06
3	.25 - .49	.250	.010	.63	.51
4	.49 - .90	.490	.127	8.05	1.14
5	.90 - 1.85	.900	.900	57.07	9.19
6	1.85 - 3.69	1.850	.486	30.82	66.27
7	3.69 - 7.42	3.690	.033	2.09	97.08
8	7.42 -14.80	7.420	.013	.82	99.18
9	14.80 -30.00	14.800	.000	.00	100.00

Mass Median Aerodynamic Diameter (MMAD): 1.54 um
Geometric standard deviation (GSD): 1.60
Number Median Aerodynamic Diameter (NMAD): .79 um
Surface Median Aerodynamic Diameter (SMAD): 1.23 um

System: BERNER-IMPACTOR I
Air flow: 5.66 liter/min.
Sampling time: 1500.00 seconds
Concentration (computed): 11.14 mg/m³ air

Respirability (percent < 1.0 um):

1. Mass related: 18.3 % (measured)
2. Number related: 69.3 % (extrapolated)

Respirability (percent < 3.0 um):

1. Mass related: 92.3 % (measured)
2. Number related: 99.1 % (extrapolated)

Respirability (percent < 5.0 um):

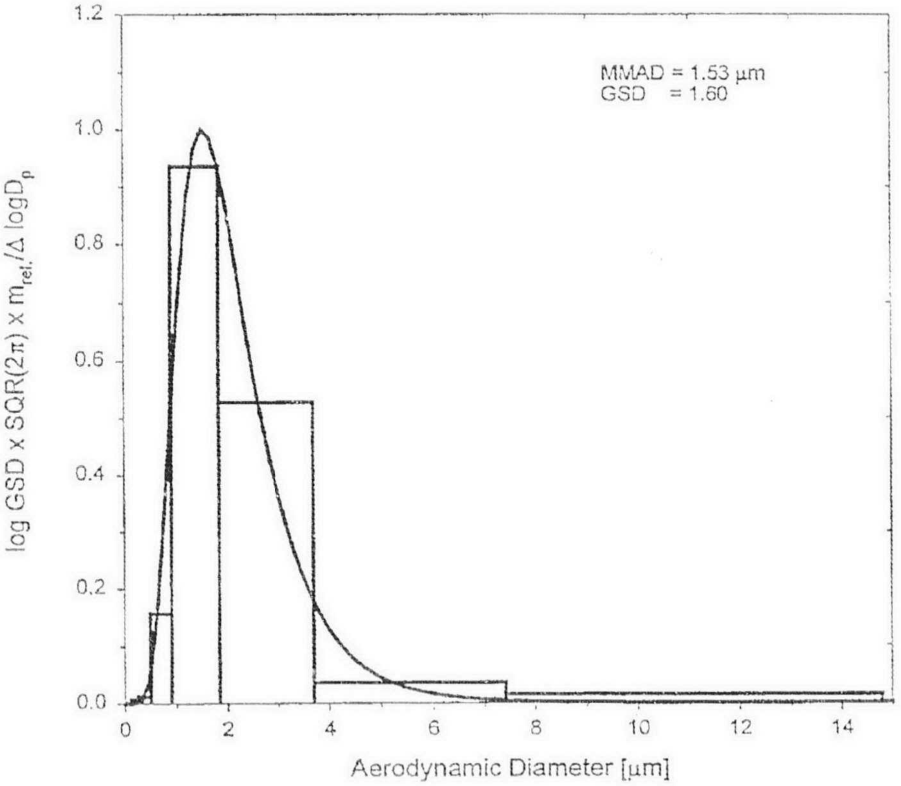
1. Mass related: 99.1 % (measured)
2. Number related: 99.1 % (extrapolated)

ECD-definition: right cut-size (Dp+1)

ll

E 10

Particle-size Distribution
Concentration: 10 mg/m³ air



E 11

ANALYSIS OF PARTICLE DISTRIBUTIONS

Type of investigation: Acute Inhalation - Aerosol

Compound: PMDI

Date of exposure: 28.01.1998 Study-no.: T7062289
Nominal concentration: 30.0 mg/m³ air

N	Impactor stage (um - um)	Cut-Off diameter (um)	Mass/ stage (mg)	Rel. mass (%)	Cumul. mass (%)
1	.06 - .12	.060	.000	.00	.00
2	.12 - .25	.120	.003	.06	.00
3	.25 - .49	.250	.017	.37	.06
4	.49 - .90	.490	.332	7.15	.43
5	.90 - 1.85	.900	2.769	59.61	7.58
6	1.85 - 3.69	1.850	1.382	29.75	67.19
7	3.69 - 7.42	3.690	.142	3.06	96.94
8	7.42 -14.80	7.420	.000	.00	100.00
9	14.80 -30.00	14.800	.000	.00	100.00

Mass Median Aerodynamic Diameter (MMAD): 1.62 um
Geometric standard deviation (GSD): 1.53
Number Median Aerodynamic Diameter (NMAD): .94 um
Surface Median Aerodynamic Diameter (SMAD): 1.35 um

System: BERNER-IMPACTOR I
Air flow: 5.66 liter/min.
Sampling time: 1500.00 seconds
Concentration (computed): 32.83 mg/m³ air

Respirability (percent < 1.0 um):

1. Mass related: 13.1 % (measured)
2. Number related: 55.7 % (extrapolated)

Respirability (percent < 3.0 um):

1. Mass related: 92.7 % (measured)
2. Number related: 99.1 % (extrapolated)

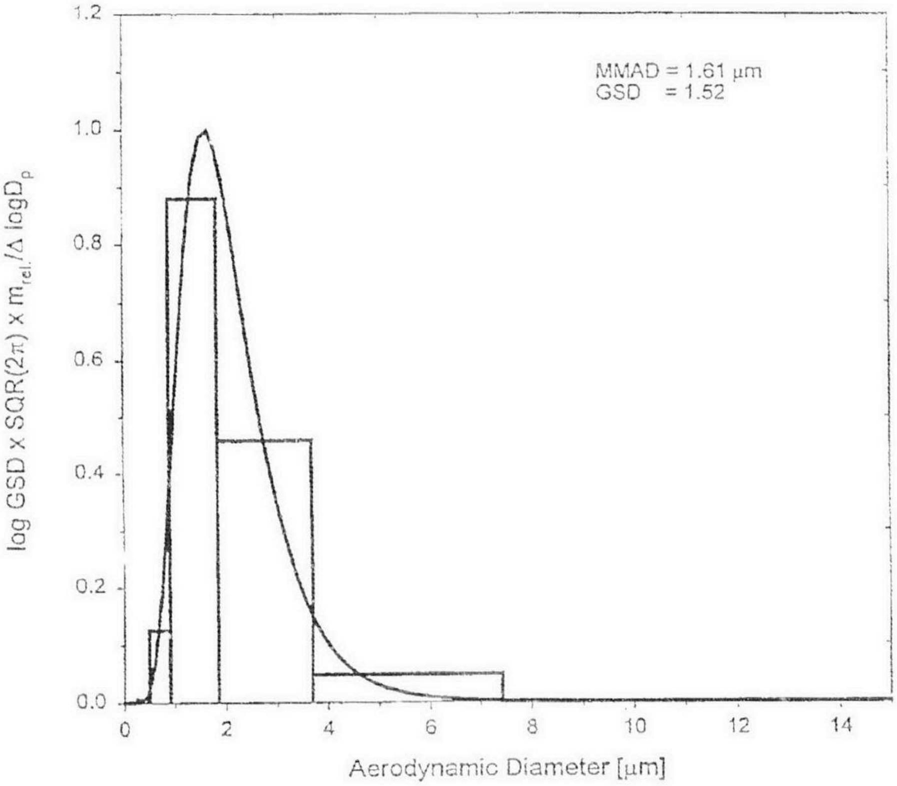
Respirability (percent < 5.0 um):

1. Mass related: 99.1 % (measured)
2. Number related: 99.1 % (extrapolated)

ECD-definition: right cut-size (Dp+1)

W

Particle-size Distribution
Concentration: 30 mg/m³ air



M

Body weights - Expos. 1 x 15 min

Analysis of Body Weights [all data in g]

Group 1: Control - FEMALES

	Postexposure Day 0	3	7	14	21
1	315.	333.	351.	373.	418.
2	320.	339.	349.	372.	408.
3	342.	358.	388.	422.	452.
4	319.	331.	366.	406.	443.
5	348.	356.	381.	410.	427.
6	341.	350.	379.	414.	440.
7	301.	315.	348.	383.	405.
8	320.	328.	339.	380.	402.
9	315.	312.	345.	366.	415.
	323.	341.	372.	392.	428.
MEAN	324.4	336.3	361.8	391.8	423.8
STD	14.7	15.7	17.5	19.9	17.1

Group 2: 3 mg/m³ air - FEMALES

	Postexposure Day 0	3	7	14	21
11	334.	352.	380.	427.	467.
12	354.	349.	390.	445.	473.
13	328.	316.	366.	400.	418.
14	323.	346.	351.	416.	457.
15	304.	343.	366.	420.	446.
16	302.	324.	347.	392.	418.
17	323.	312.	364.	378.	431.
18	304.	268.	321.	340.	389.
19	312.	298.	356.	381.	427.
20	329.	294.	364.	392.	427.
MEAN	321.3	320.2	360.5	339.1	435.3
STD	16.3	27.9	18.8	29.8	25.6

Group 3: 10 mg/m³ air - FEMALES

	Postexposure Day 0	3	7	14	21
21	340.	359.	377.	434.	486.
22	362.	381.	403.	444.	460.
23	353.	370.	380.	456.	499.
24	306.	326.	342.	392.	410.
25	324.	350.	369.	414.	443.
26	340.	342.	367.	401.	402.
27	347.	358.	387.	426.	459.
28	314.	332.	353.	390.	424.
29	319.	330.	339.	377.	390.
30	342.	356.	369.	406.	431.
MEAN	334.7	350.4	368.6	414.0	440.4
STD	13.1	18.0	19.9	25.5	35.8

ll

Group 4: 30 mg/m³ air - FEMALES

	Postexposure Day				
	0	3	7	14	21
31	323.	340.	366.	421.	455.
32	311.	333.	342.	374.	398.
33	333.	345.	369.	402.	439.
34	345.	366.	385.	439.	483.
35	362.	391.	420.	502.	533.
36	331.	352.	379.	434.	462.
37	326.	338.	361.	408.	453.
38	323.	339.	379.	425.	463.
39	315.	330.	354.	377.	404.
40	354.	377.	408.	459.	506.
MEAN	332.3	351.1	376.3	424.1	459.6
STD	16.6	20.4	23.7	38.1	41.5

ll

F 01

Analysis of Body Weight Gains [all data in g]
Group 1: Control - FEMALES

	Postexposure Day 3	7	14	21
1	18.00	18.00	22.00	45.00
2	19.00	10.00	23.00	36.00
3	16.00	30.00	34.00	30.00
4	12.00	35.00	40.00	37.00
5	8.00	25.00	29.00	17.00
6	9.00	29.00	35.00	26.00
7	14.00	33.00	35.00	22.00
8	8.00	11.00	41.00	22.00
9	-3.00	33.00	21.00	49.00
10	18.00	31.00	20.00	36.00
MEAN	11.9	25.5	30.0	32.0
STD	6.7	9.3	8.0	10.4

Group 2: 3 mg/m³ air - FEMALES

	Postexposure Day 3	7	14	21
11	18.00	28.00	47.00	40.00
12	-5.00	41.00	55.00	28.00
13	-12.00	50.00	34.00	18.00
14	23.00	5.00	65.00	41.00
15	39.00	23.00	54.00	26.00
16	22.00	23.00	45.00	26.00
17	-11.00	52.00	14.00	53.00
18	-36.00	53.00	19.00	49.00
19	-14.00	58.00	25.00	46.00
20	-35.00	70.00	28.00	35.00
MEAN	-1.1	40.3	38.6	36.2
STD	25.5	20.0	17.1	11.5

Group 3: 10 mg/m³ air - FEMALES

	Postexposure Day 3	7	14	21
21	19.00	18.00	57.00	52.00
22	19.00	22.00	41.00	16.00
23	17.00	10.00	76.00	43.00
24	20.00	16.00	50.00	18.00
25	26.00	19.00	45.00	29.00
26	2.00	25.00	34.00	1.00
27	11.00	29.00	39.00	33.00
28	18.00	21.00	37.00	34.00
29	11.00	9.00	38.00	13.00
30	14.00	13.00	37.00	25.00
MEAN	15.7	18.2	45.4	26.4
STD	6.6	6.4	12.8	15.1

Group 4: 30 mg/m³ air - FEMALES

	Postexposure Day 3	7	14	21
31	17.00	26.00	55.00	34.00
32	22.00	9.00	32.00	24.00
33	12.00	24.00	33.00	37.00
34	21.00	19.00	54.00	44.00
35	29.00	29.00	82.00	31.00
36	21.00	27.00	55.00	28.00
37	12.00	23.00	47.00	45.00
38	16.00	40.00	46.00	38.00
39	15.00	24.00	23.00	27.00
40	23.00	31.00	51.00	47.00
MEAN	18.8	25.2	47.8	35.5
STD	5.4	8.0	16.4	8.1

ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of Day: 3 / FEMALES

Group-no.: 1

18.000	19.000	16.000	12.000	8.000
9.000	14.000	8.000	-3.000	18.000

MEDIAN= 13.000 MEAN= 11.900 STD = 6.724

Group-no.: 2

18.000	-5.000	-12.000	23 000	39.000
22.000	-11.000	-36.000	-14.000	-35.000

MEDIAN= -8.000 MEAN= -1.100 STD = 25.501

Group-no.: 3

19.000	19.000	17.000	20.000	26.000
2.000	11.000	18.000	11.000	14.000

MEDIAN= 17.500 MEAN= 15.700 STD = 6.567

Group-no.: 4

17.000	22.000	12.000	21.000	29.000
21.000	12.000	16.000	15.000	23.000

MEDIAN= 19.000 MEAN= 18.800 STD = 5.371

BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

CALCULATED F	D.F.s	PROBABILITY
10.4849	3 & 2333.	.0000

HETEROGENEOUS VARIANCES (ONE-TAILED TEST)

ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB
TREATMENT	2297.	3	765.76	3.991	.015
ERROR	6908.	36	191.88		
TOTAL	9205.	39			

OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL

Ne

GAMES AND HOWELL MODIFICATION OF
TUKEY-KRAMERs HONESTLY SIGNIFICANT DIFFERENCE TEST
(WITH THE STUDENTIZED RANGE STATISTIC)

GROUPS COMPARED	CALCULATED TEST VALUE	DEGREES OF FREEDOM	PROBABILITY	CONCLUSION
5. % ONE-TAILED TEST				
1 AND 2	-2.20	10	.4417	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 2	2.20	10	.4417	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
1 AND 3	1.81	18	.5875	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 3	1.81	18	.5875	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
1 AND 4	3.59	17	.0898	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 4	3.59	17	.0898	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
2 AND 3	2.85	10	.2446	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
2 AND 3	2.85	10	.2446	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
2 AND 4	3.41	10	.1369	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
2 AND 4	3.41	10	.1369	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
3 AND 4	1.63	17	.6616	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
3 AND 4	1.63	17	.6616	NOT SIGNIFICANT

Al

ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of Day: 7 / FEMALES

Group-no.: 1

18.000	10.000	30.000	35.000	25.000
29.000	33.000	11.000	33.000	31.000

MEDIAN= 29.500 MEAN= 25.500 STD = 9.265

Group-no.: 2

28.000	41.000	50.000	5.000	23.000
23.000	52.000	53.000	58.000	70.000

MEDIAN= 45.500 MEAN= 40.300 STD = 19.956

Group-no.: 3

18.000	22.000	10.000	16.000	19.000
25.000	29.000	21.000	9.000	13.000

MEDIAN= 18.500 MEAN= 18.200 STD = 6.408

Group-no.: 4

26.000	9.000	24.000	19.000	29.000
27.000	23.000	40.000	24.000	31.000

MEDIAN= 25.000 MEAN= 25.200 STD = 8.025

BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

CALCULATED F	D.F.s	PROBABILITY
4.7609	3 & 2333.	.0030

HETEROGENEOUS VARIANCES (ONE-TAILED TEST)

ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB
TREATMENT	2595.	3	864.87	5.868	.003
ERROR	5306.	36	147.38		
TOTAL	7900.	39			

OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL

N

GAMES AND HOWELL MODIFICATION OF
TUKEY-KRAMERs HONESTLY SIGNIFICANT DIFFERENCE TEST
(WITH THE STUDENTIZED RANGE STATISTIC)

GROUPS COMPARED	CALCULATED TEST VALUE	DEGREES OF FREEDOM	PROBABILITY	CONCLUSION
5. % ONE-TAILED TEST				
1 AND 2	3.01	13	.1956	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 2	3.01	13	.1956	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
1 AND 3	-2.90	16	.2117	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 3	2.90	16	.2117	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
1 AND 4	-.11	18	.9998	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 4	.11	18	.9998	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
2 AND 3	-4.72	11	.0291	SIGNIFICANT
5. % TWO-TAILED TEST				
2 AND 3	4.72	11	.0291	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
2 AND 4	-3.14	12	.1729	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
2 AND 4	3.14	12	.1729	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
3 AND 4	3.05	17	.1758	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
3 AND 4	3.05	17	.1758	NOT SIGNIFICANT

Al

ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of Day: 14 / FEMALES

Group-no.: 1

22.000	23.000	34.000	40.000	29.000
35.000	35.000	41.000	21.000	20.000

MEDIAN= 31.500 MEAN= 30.000 STD = 8.042

Group-no.: 2

47.000	55.000	34.000	65.000	54.000
45.000	14.000	19.000	25.000	28.000

MEDIAN= 39.500 MEAN= 38.600 STD = 17.070

Group-no.: 3

57.000	41.000	76.000	50.000	45.000
34.000	39.000	37.000	38.000	37.000

MEDIAN= 40.000 MEAN= 45.400 STD = 12.817

Group-no.: 4

55.000	32.000	33.000	54.000	82.000
55.000	47.000	46.000	23.000	51.000

MEDIAN= 49.000 MEAN= 47.800 STD = 16.363

BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

CALCULATED F	D.F.s	PROBABILITY
1.7154	3 & 2333.	.1601

HOMOGENEOUS VARIANCES (ONE-TAILED TEST)

ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB
TREATMENT	1912.	3	637.17	3.234	.033
ERROR	7092.	36	197.01		
TOTAL	9004.	39			

OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL

nl

GAMES AND HOWELL MODIFICATION OF
TUKEY-KRAMERs HONESTLY SIGNIFICANT DIFFERENCE TEST
(WITH THE STUDENTIZED RANGE STATISTIC)

GROUPS COMPARED	CALCULATED TEST VALUE	DEGREES OF FREEDOM	PROBABILITY	CONCLUSION
5. % ONE-TAILED TEST				
1 AND 2	2.04	13	.4976	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 2	2.04	13	.4976	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
1 AND 3	4.55	15	.0263	SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 3	4.55	15	.0263	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
1 AND 4	4.37	13	.0381	SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 4	4.37	13	.0381	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
2 AND 3	1.42	17	.7473	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
2 AND 3	1.42	17	.7473	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
2 AND 4	1.74	18	.6164	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
2 AND 4	1.74	18	.6164	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
3 AND 4	.52	17	.9828	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
3 AND 4	.52	17	.9828	NOT SIGNIFICANT

hl

ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of Day: 21 / FEMALES

Group-no.: 1

45.000	36.000	30.000	37.000	17.000
26.000	22.000	22.000	49.000	36.000

MEDIAN= 33.000 MEAN= 32.000 STD = 10.435

Group-no.: 2

40.000	28.000	18.000	41.000	26.000
26.000	53.000	49.000	46.000	35.000

MEDIAN= 37.500 MEAN= 36.200 STD = 11.487

Group-no.: 3

52.000	16.000	43.000	18.000	29.000
1.000	33.000	34.000	13.000	25.000

MEDIAN= 27.000 MEAN= 26.400 STD = 15.072

Group-no.: 4

34.000	24.000	37.000	44.000	31.000
28.000	45.000	38.000	27.000	47.000

MEDIAN= 35.500 MEAN= 35.500 STD = 8.073

BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

CALCULATED F	D.F.s	PROBABILITY
1.1322	3 & 2333.	.3346

HOMOGENEOUS VARIANCES (ONE-TAILED TEST)

ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB
TREATMENT	601.5	3	200.49	1.504	.229
ERROR	4799.	36	133.29		
TOTAL	5400.	39			

NO OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL
NO STATISTICAL DIFFERENCE BETWEEN THE GROUPS

N

F 10

Body weights - Expos. 1 x 1 hr

Analysis of Body Weights [all data in g]

Group 1: Control - FEMALES

	Postexposure Day 0	3	7	14	21
41	308.	322.	339.	365.	390.
42	315.	346.	356.	395.	412.
43	312.	320.	341.	367.	399.
44	295.	308.	333.	359.	384.
45	342.	351.	373.	387.	417.
46	319.	333.	353.	385.	404.
47	277.	311.	332.	366.	401.
48	301.	322.	344.	357.	383.
49	322.	332.	350.	383.	411.
50	276.	307.	330.	363.	406.
MEAN	306.7	325.2	345.1	372.7	400.7
STD	20.3	15.2	13.3	13.4	11.8

Group 2: 3 mg/m³ air - FEMALES

	Postexposure Day 0	3	7	14	21
51	328.	350.	376.	419.	468.
52	329.	350.	377.	421.	468.
53	333.	352.	379.	415.	443.
54	329.	355.	383.	437.	459.
55	324.	346.	377.	432.	493.
56	343.	372.	415.	477.	526.
57	314.	335.	361.	375.	424.
58	314.	337.	354.	370.	410.
59	332.	356.	385.	413.	474.
60	301.	320.	349.	359.	391.
MEAN	324.7	347.3	375.6	411.8	455.6
STD	12.0	14.1	18.6	35.5	40.0

Group 3: 10 mg/m³ air - FEMALES

	Postexposure Day 0	3	7	14	21
61	334.	345.	381.	405.	455.
62	352.	367.	401.	438.	469.
63	306.	321.	341.	376.	404.
64	328.	338.	358.	380.	438.
65	349.	370.	403.	449.	495.
66	353.	360.	385.	447.	486.
67	314.	342.	381.	410.	454.
68	325.	343.	380.	396.	440.
69	314.	331.	357.	370.	392.
70	315.	332.	365.	376.	421.
MEAN	329.0	344.9	375.2	404.7	445.4
STD	17.4	16.1	19.8	30.6	33.5

Group 4: 30 mg/m³ air - FEMALES

	Postexposure Day				
	0	3	7	14	21
71	326.	331.	356.	396.	411.
72	331.	342.	371.	389.	420.
73	328.	349.	374.	416.	448.
74	335.	364.	377.	404.	449.
75	350.	363.	402.	434.	476.
76	349.	362.	385.	416.	445.
77	321.	331.	340.	364.	382.
78	355.	373.	385.	442.	479.
79	352.	363.	373.	402.	434.
80	326.	348.	370.	406.	447.
MEAN	337.3	352.6	373.3	406.9	439.1
STD	12.8	14.6	16.8	22.2	29.2

Analysis of Body Weight Gains [all data in g]
Group 1: Control - FEMALES

	Postexposure Day 3	7	14	21
41	14.00	17.00	26.00	25.00
42	31.00	10.00	39.00	17.00
43	8.00	21.00	26.00	32.00
44	13.00	25.00	26.00	25.00
45	9.00	22.00	14.00	30.00
46	14.00	20.00	32.00	19.00
47	34.00	21.00	34.00	35.00
48	21.00	22.00	13.00	26.00
49	10.00	18.00	33.00	28.00
50	31.00	23.00	33.00	43.00
MEAN	18.5	19.9	27.6	28.0
STD	10.0	4.2	8.5	7.6

Group 2: 3 mg/m³ air - FEMALES

	Postexposure Day 3	7	14	21
51	22.00	26.00	43.00	49.00
52	21.00	27.00	44.00	47.00
53	19.00	27.00	36.00	28.00
54	26.00	28.00	54.00	22.00
55	22.00	31.00	55.00	61.00
56	29.00	43.00	62.00	49.00
57	21.00	26.00	14.00	49.00
58	23.00	17.00	16.00	40.00
59	24.00	29.00	28.00	61.00
60	19.00	29.00	10.00	32.00
MEAN	22.6	28.3	36.2	43.8
STD	3.1	6.4	18.6	13.2

Group 3: 10 mg/m³ air - FEMALES

	Postexposure Day 3	7	14	21
61	11.00	36.00	24.00	50.00
62	15.00	34.00	37.00	31.00
63	15.00	20.00	35.00	28.00
64	10.00	20.00	22.00	58.00
65	21.00	33.00	46.00	46.00
66	7.00	25.00	62.00	39.00
67	28.00	39.00	29.00	44.00
68	18.00	37.00	16.00	44.00
69	17.00	26.00	13.00	22.00
70	17.00	33.00	11.00	45.00
MEAN	15.9	30.3	29.5	40.7
STD	6.0	7.0	16.0	10.9

Group 4: 30 mg/m³ air - FEMALES

	Postexposure Day			
	3	7	14	21
71	5.00	25.00	40.00	15.00
72	11.00	29.00	18.00	31.00
73	21.00	25.00	42.00	32.00
74	29.00	13.00	27.00	45.00
75	13.00	39.00	32.00	42.00
76	13.00	23.00	31.00	29.00
77	10.00	9.00	24.00	18.00
78	18.00	12.00	57.00	37.00
79	11.00	10.00	29.00	32.00
80	22.00	22.00	36.00	41.00
MEAN	15.3	20.7	33.6	32.2
STD	7.1	9.6	10.9	9.8

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ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of Day: 3 / FEMALES

Group-no.: 1

14.000	31.000	8.000	13.000	9.000
14.000	34.000	21.000	10.000	31.000

MEDIAN= 14.000 MEAN= 18.500 STD = 10.014

Group-no.: 2

22.000	21.000	19.000	26.000	22.000
29.000	21.000	23.000	24.000	19.000

MEDIAN= 22.000 MEAN= 22.600 STD = 3.098

Group-no.: 3

11.000	15.000	15.000	10.000	21.000
7.000	28.000	18.000	17.000	17.000

MEDIAN= 16.000 MEAN= 15.900 STD = 5.953

Group-no.: 4

5.000	11.000	21.000	29.000	13.000
13.000	10.000	18.000	11.000	22.000

MEDIAN= 13.000 MEAN= 15.300 STD = 7.103

BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

CALCULATED F	D.F.s	PROBABILITY
3.4634	3 & 2333.	.0156

HETEROGENEOUS VARIANCES (ONE-TAILED TEST)

ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB
TREATMENT	330.9	3	110.29	2.254	.098
ERROR	1762.	36	48.942		
TOTAL	2093.	39			

NO OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL
NO STATISTICAL DIFFERENCE BETWEEN THE GROUPS

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ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of Day: 7 / FEMALES

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 Group-no.:  1
     17.000       10.000       21.000       25.000       22.000
     20.000       21.000       22.000       18.000       23.000
   MEDIAN=    21.000   MEAN=     19.900   STD =      4.175

 Group-no.:  2
     26.000       27.000       27.000       28.000       31.000
     43.000       26.000       17.000       29.000       29.000
   MEDIAN=    27.500   MEAN=     28.300   STD =      6.378

 Group-no.:  3
     36.000       34.000       20.000       20.000       33.000
     25.000       39.000       37.000       26.000       33.000
   MEDIAN=    33.000   MEAN=     30.300   STD =      6.993

 Group-no.:  4
     25.000       29.000       25.000       13.000       39.000
     23.000        9.000       12.000       10.000       22.000
   MEDIAN=    22.500   MEAN=     20.700   STD =      9.627
```

BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

CALCULATED F	D.F.s	PROBABILITY
1.8914	3 & 2333.	.1273

HOMOGENEOUS VARIANCES (ONE-TAILED TEST)

ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB
TREATMENT	833.2	3	277.73	5.563	.003
ERROR	1797.	36	49.922		
TOTAL	2630.	39			

OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL

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GAMES AND HOWELL MODIFICATION OF
TUKEY-KRAMERs HONESTLY SIGNIFICANT DIFFERENCE TEST
(WITH THE STUDENTIZED RANGE STATISTIC)

GROUPS COMPARED	CALCULATED TEST VALUE	DEGREES OF FREEDOM	PROBABILITY	CONCLUSION
5. % ONE-TAILED TEST				
1 AND 2	4.93	16	.0146	SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 2	4.93	16	.0146	SIGNIFICANT
5. % ONE-TAILED TEST				
1 AND 3	5.71	15	.0053	SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 3	5.71	15	.0053	SIGNIFICANT
5. % ONE-TAILED TEST				
1 AND 4	.34	12	.9948	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 4	.34	12	.9948	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
2 AND 3	.95	18	.9077	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
2 AND 3	.95	18	.9077	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
2 AND 4	-2.94	16	.2011	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
2 AND 4	2.94	16	.2011	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
3 AND 4	-3.61	16	.0893	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
3 AND 4	3.61	16	.0893	NOT SIGNIFICANT

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ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of Day: 14 / FEMALES

Group-no.: 1

26.000	39.000	26.000	26.000	14.000
32.000	34.000	13.000	33.000	33.000

MEDIAN= 29.000 MEAN= 27.600 STD = 8.527

Group-no.: 2

43.000	44.000	36.000	54.000	55.000
62.000	14.000	16.000	28.000	10.000

MEDIAN= 39.500 MEAN= 200 STD = 18.552

Group-no.: 3

24.000	37.000	35.000	22.000	46.000
62.000	29.000	16.000	13.000	11.000

MEDIAN= 26.500 MEAN= 29.500 STD = 15.981

Group-no.: 4

40.000	18.000	42.000	27.000	32.000
31.000	24.000	57.000	29.000	36.000

MEDIAN= 31.500 MEAN= 33.600 STD = 10.926

BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

CALCULATED F	D.F.s	PROBABILITY
2.0057	3 & 2333.	.1095

HOMOGENEOUS VARIANCES (ONE-TAILED TEST)

ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB
TREATMENT	455.1	3	151.69	.766	.523
ERROR	7125.	36	197.91		
TOTAL	7580.	39			

NO OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL
NO STATISTICAL DIFFERENCE BETWEEN THE GROUPS

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ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of Day: 21 / FEMALES

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Group-no.:  1
     25.000       17.000       32.000       25.000       30.000
     19.000       35.000       26.000       23.000       43.000
   MEDIAN=     27.000   MEAN=     28.000   STD =     7.587

Group-no.:  2
     49.000       47.000       28.000       22.000       61.000
     49.000       49.000       40.000       61.000       32.000
   MEDIAN=     48.000   MEAN=     43.800   STD =    13.172

Group-no.:  3
     50.000       31.000       28.000       58.000       46.000
     39.000       44.000       44.000       22.000       45.000
   MEDIAN=     44.000   MEAN=     40.700   STD =    10.863

Group-no.:  4
     15.000       31.000       32.000       45.000       42.000
     29.000       18.000       37.000       32.000       41.000
   MEDIAN=     32.000   MEAN=     32.200   STD =     9.830
```

BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

CALCULATED F	D.F.s	PROBABILITY
.8682	3 & 2333.	.5406

HOMOGENEOUS VARIANCES (ONE-TAILED TEST)

ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB.
TREATMENT	1612.	3	537.49	4.824	.007
ERROR	4011.	36	111.42		
TOTAL	5624.	39			

OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL

GAMES AND HOWELL MODIFICATION OF
TUKEY-KRAMERs HONESTLY SIGNIFICANT DIFFERENCE TEST
(WITH THE STUDENTIZED RANGE STATISTIC)

GROUPS COMPARED	CALCULATED TEST VALUE	DEGREES OF FREEDOM	PROBABILITY	CONCLUSION
5. % ONE-TAILED TEST				
1 AND 2	4.65	14	.0246	SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 2	4.65	14	.0246	SIGNIFICANT
5. % ONE-TAILED TEST				
1 AND 3	4.29	16	.0360	SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 3	4.29	16	.0360	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
1 AND 4	1.51	17	.7119	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 4	1.51	17	.7119	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
2 AND 3	-.81	17	.9385	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
2 AND 3	.81	17	.9385	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
2 AND 4	-3.16	17	.1545	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
2 AND 4	3.16	17	.1545	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
3 AND 4	-2.59	18	.2901	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
3 AND 4	2.59	18	.2901	NOT SIGNIFICANT

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Body Weights - Expos.: 1 x 6 hrs

Analysis of Body Weights [all data in g]

Group 1: Control - FEMALES

	Postexposure Day 0	3	7	14	21
81	289.	306.	334.	367.	407.
82	321.	336.	370.	385.	426.
83	284.	297.	324.	358.	402.
84	313.	331.	354.	397.	408.
85	264.	277.	315.	348.	369.
86	280.	294.	325.	358.	392.
87	286.	303.	338.	369.	403.
88	315.	344.	388.	430.	465.
89	319.	338.	369.	406.	437.
90	316.	339.	359.	402.	436.
MEAN	298.7	316.5	347.6	382.0	414.5
STD	20.3	23.7	24.0	26.3	27.1

Group 2: 1 mg/m³ air - FEMALES

	Postexposure Day 0	3	7	14	21
91	368.	387.	432.	477.	507.
92	355.	369.	404.	452.	482.
93	302.	311.	340.	375.	420.
94	303.	336.	369.	406.	446.
95	330.	345.	385.	438.	468.
96	294.	317.	366.	395.	463.
97	320.	334.	368.	408.	446.
98	311.	329.	358.	396.	456.
99	324.	332.	365.	396.	444.
100	316.	327.	372.	427.	484.
MEAN	322.3	338.7	375.9	417.0	461.6
STD	23.5	23.2	25.8	31.1	24.9

Group 3: 3 mg/m³ air - FEMALES

	Postexposure Day 0	3	7	14	21
101	336.	342.	368.	420.	435.
102	289.	307.	332.	380.	426.
103	272.	289.	306.	354.	379.
104	321.	334.	364.	389.	422.
105	293.	319.	345.	402.	466.
106	324.	334.	359.	386.	403.
107	296.	318.	336.	336.	407.
108	300.	322.	349.	366.	410.
109	353.	371.	388.	435.	481.
110	349.	364.	389.	427.	498.
MEAN	313.3	330.0	353.6	389.5	432.7
STD	27.3	24.8	25.6	32.2	37.7

Group 4: 10 mg/m³ air - FEMALES

	Postexposure Day				
	0	3	7	14	21
111	292.	305.	338.	376.	410.
112	291.	303.	326.	374.	424.
113	306.	313.	339.	372.	404.
114	300.	308.	340.	369.	415.
115	306.	320.	332.	380.	418.
116	319.	329.	359.	397.	429.
117	306.	331.	358.	387.	428.
118	348.	370.	394.	429.	469.
119	318.	348.	356.	401.	437.
120	297.	320.	341.	378.	411.
MEAN	308.3	324.7	348.3	386.3	424.5
STD	16.9	21.0	19.5	18.3	18.6

G 08

Analysis of Body Weight Gains [all data in g]

Group 1: Control - FEMALES

	Postexposure Day 3	7	14	21
81	17.00	28.00	33.00	40.00
82	15.00	34.00	15.00	41.00
83	13.00	27.00	34.00	44.00
84	18.00	23.00	43.00	11.00
85	13.00	38.00	33.00	21.00
86	14.00	31.00	33.00	34.00
87	17.00	35.00	31.00	34.00
88	29.00	44.00	42.00	35.00
89	19.00	31.00	37.00	31.00
90	23.00	20.00	43.00	34.00
MEAN	17.8	31.1	34.4	32.5
STD	5.0	7.1	8.2	9.8

Group 2: 1 mg/m³ air FEMALES

	Postexposure Day 3	7	14	21
91	19.00	45.00	45.00	30.00
92	14.00	35.00	48.00	30.00
93	9.00	29.00	35.00	45.00
94	33.00	33.00	37.00	40.00
95	15.00	40.00	53.00	30.00
96	23.00	49.00	29.00	68.00
97	14.00	34.00	40.00	38.00
98	18.00	29.00	38.00	60.00
99	8.00	33.00	31.00	48.00
100	11.00	45.00	55.00	57.00
MEAN	16.4	37.2	41.1	44.6
STD	7.4	7.1	8.9	13.6

Group 3: 3 mg/m³ air - FEMALES

	Postexposure Day 3	7	14	21
101	6.00	26.00	52.00	15.00
102	18.00	25.00	48.00	46.00
103	17.00	17.00	48.00	25.00
104	13.00	30.00	25.00	33.00
105	26.00	26.00	57.00	64.00
106	10.00	25.00	27.00	17.00
107	22.00	18.00	.00	71.00
108	22.00	27.00	17.00	44.00
109	18.00	17.00	47.00	46.00
110	15.00	25.00	38.00	71.00
MEAN	16.7	23.6	35.9	43.2
STD	6.0	4.6	18.2	20.8

Group 4: 10 mg/m³ air - FEMALES

	Postexposure Day 3	7	14	21
111	13.00	33.00	38.00	34.00
112	12.00	23.00	48.00	50.00
113	7.00	26.00	33.00	32.00
114	8.00	32.00	29.00	46.00
115	14.00	12.00	48.00	38.00
116	10.00	30.00	38.00	32.00
117	25.00	27.00	29.00	41.00
118	22.00	24.00	35.00	40.00
119	30.00	8.00	45.00	36.00
120	23.00	21.00	37.00	33.00
MEAN	16.4	23.6	38.0	38.2
STD	8.0	8.2	7.0	6.1

ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of Day: 3 / FEMALES

Group-no.: 1

17.000	15.000	13.000	18.000	13.000
14.000	17.000	29.000	19.000	23.000

MEDIAN= 17.000 MEAN= 17.800 STD = 4.984

Group-no.: 2

19.000	14.000	9.000	33.000	15.000
23.000	14.000	18.000	8.000	11.000

MEDIAN= 14.500 MEAN= 16.400 STD = 7.427

Group-no.: 3

6.000	18.000	17.000	13.000	26.000
10.000	22.000	22.000	18.000	15.000

MEDIAN= 17.500 MEAN= 16.700 STD = 5.980

Group-no.: 4

13.000	12.000	7.000	8.000	14.000
10.000	25.000	22.000	30.000	23.000

MEDIAN= 13.500 MEAN= 16.400 STD = 7.961

BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

CALCULATED F	D.F.s	PROBABILITY
.7893	3 & 2333.	.5027

HOMOGENEOUS VARIANCES (ONE-TAILED TEST)

ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB
TREATMENT	13.08	3	4.3583	.099	.959
ERROR	1589.	36	44.147		
TOTAL	1602.	39			

NO OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL
NO STATISTICAL DIFFERENCE BETWEEN THE GROUPS

ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of Day: 7 / FEMALES

Group-no.: 1

28.000	34.000	27.000	23.000	38.000
31.000	35.000	44.000	31.000	20.000

MEDIAN= 31.000 MEAN= 31.100 STD = 7.094

Group-no.: 2

45.000	35.000	29.000	33.000	40.000
49.000	34.000	29.000	33.000	45.000

MEDIAN= 34.500 MEAN= 37.200 STD = 7.099

Group-no.: 3

26.000	25.000	17.000	30.000	26.000
25.000	18.000	27.000	17.000	25.000

MEDIAN= 25.000 MEAN= 23.600 STD = 4.600

Group-no.: 4

33.000	23.000	26.000	32.000	12.000
30.000	27.000	24.000	8.000	21.000

MEDIAN= 25.000 MEAN= 23.600 STD = 8.181

BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

CALCULATED F	D.F.s	PROBABILITY
.9622	3 & 2333.	.5888

HOMOGENEOUS VARIANCES (ONE-TAILED TEST)

ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB
TREATMENT	1320.	3	440.16	9.347	.000
ERROR	1695.	36	47.092		
TOTAL	3016.	39			

OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL

GAMES AND HOWELL MODIFICATION OF
TUKEY-KRAMERs HONESTLY SIGNIFICANT DIFFERENCE TEST
(WITH THE STUDENTIZED RANGE STATISTIC)

GROUPS COMPARED	CALCULATED TEST VALUE	DEGREES OF FREEDOM	PROBABILITY	CONCLUSION
5. % ONE-TAILED TEST				
1 AND 2	2.72	18	.2540	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 2	2.72	18	.2540	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
1 AND 3	-4.09	15	.0494	SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 3	4.09	15	.0494	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
1 AND 4	-3.10	18	.1637	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 4	3.10	18	.1637	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
2 AND 3	-7.32	15	.0006	SIGNIFICANT
5. % TWO-TAILED TEST				
2 AND 3	7.32	15	.0006	SIGNIFICANT
5. % ONE-TAILED TEST				
2 AND 4	-5.61	18	.0045	SIGNIFICANT
5. % TWO-TAILED TEST				
2 AND 4	5.61	18	.0045	SIGNIFICANT
5. % ONE-TAILED TEST				
3 AND 4	.10	14	.9999	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
3 AND 4	.10	14	.9999	NOT SIGNIFICANT

G 13

ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of Day: 14 / FEMALES

Group-no.: 1

33.000	15.000	34.000	43.000	33.000
33.000	31.000	42.000	37.000	43.000

MEDIAN= 33.500 MEAN= 34.400 STD = 8.208

Group-no.: 2

45.000	48.000	35.000	37.000	53.000
29.000	40.000	38.000	31.000	55.000

MEDIAN= 39.000 MEAN= 41.100 STD = 8.888

Group-no.: 3

52.000	48.000	48.000	25.000	57.000
27.000	.000	17.000	47.000	38.000

MEDIAN= 42.500 MEAN= 35.900 STD = 18.163

Group-no.: 4

38.000	48.000	33.000	29.000	48.000
38.000	29.000	35.000	45.000	37.000

MEDIAN= 37.500 MEAN= 38.000 STD = 7.040

BOX'S TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

CALCULATED F	D.F.s	PROBABILITY
3.5472	3 & 2333.	.0139

HETEROGENEOUS VARIANCES (ONE-TAILED TEST)

ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB
TREATMENT	252.9	3	84.300	.641	.597
ERROR	4732.	36	131.45		
TOTAL	4985.	39			

NO OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL
NO STATISTICAL DIFFERENCE BETWEEN THE GROUPS

CONT:

ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of Day: 21 / FEMALES

Group-no.: 1

40.000	41.000	44.000	11.000	21.000
34.000	34.000	35.000	31.000	34.000

MEDIAN= 34.000 MEAN= 32.500 STD = 9.835

Group-no.: 2

30.000	30.000	45.000	40.000	30.000
68.000	38.000	60.000	48.000	57.000

MEDIAN= 42.500 MEAN= 44.600 STD = 13.558

Group-no.: 3

15.000	46.000	25.000	33.000	64.000
17.000	71.000	44.000	46.000	71.000

MEDIAN= 45.000 MEAN= 43.200 STD = 20.848

Group-no.: 4

34.000	50.000	32.000	46.000	38.000
32.000	41.000	40.000	36.000	33.000

MEDIAN= 37.000 MEAN= 38.200 STD = 6.125

BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

CALCULATED F	D.F.s	PROBABILITY
4.2164	3 & 2333.	.0059

HETEROGENEOUS VARIANCES (ONE-TAILED TEST)

ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB
TREATMENT	903.3	3	301.09	1.600	.205
ERROR	6774.	36	188.17		
TOTAL	7677.	39			

NO OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL
NO STATISTICAL DIFFERENCE BETWEEN THE GROUPS

Lung Weights - Expos.: 1 x 15 min

```
          ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA
------------------------------------------------------------------
Analysis of LUNG WEIGHT¹ - absolute/FEMALES
------------------------------------------------------------------

 Group-no.:  1 (1-10)
    2644.000    2476.000    2823.000    2776.000    2763.000
    3104.000    2406.000    2474.000    2341.000    2441.000
    MEDIAN=  2560.000   MEAN=  2624.800    STD =   239.983

 Group-no.:  2 (11-20)
    3052.000    2707.000    2576.000    3200.000    2563.000
    2650.000    2696.000    2675.000    2692.000    2627.000
    MEDIAN=  2683.500   MEAN=  2743.800    STD =   210.145

 Group-no.:  3 (21-30)
    3007.000    2848.000    3130.000    2655.000    2636.000
    2412.000    2844.000    2735.000    2536.000    2530.000
    MEDIAN=  2695.000   MEAN=  2733.300    STD =   225.287

 Group-no.:  4 (31-40)
    2540.000    2568.000    2474.000    3338.000    3101.000
    3133.000    2590.000    2678.000    2583.000    3091.000
    MEDIAN=  2634.000   MEAN=  2809.600    STD =   317.712
------------------------------------------------------------------
   BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

  CALCULATED F        D.F.s              PROBABILITY
  ------------    --------------         ------------
     .6096           3 &   2333.            .6129

   HOMOGENEOUS VARIANCES (ONE-TAILED TEST)
------------------------------------------------------------------

   ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE
------------------------------------------------------------------
 SOURCE          SS          DF        MS           F         PROB
------------------------------------------------------------------
 TREATMENT   1.7586E+05       3      58622.        .925       .560
 ERROR       2.2810E+06      36      63362.
 TOTAL       2.4569E+06      39
------------------------------------------------------------------
   NO OVERALL SIGNIFICANCE AT  5.% (ONE-TAILED) LEVEL
   NO STATISTICAL DIFFERENCE BETWEEN THE GROUPS
```

[1] Unit of lung weights: mg

ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of LUNG WEIGHT[2] - relative to BODY WEIGHT/FEMALES

Group-no.: 1 (1-10)

634.053	612.871	605.794	619.643	661.005
703.855	588.264	586.256	560.048	566.357
MEDIAN= 609.333	MEAN= 613.815	STD = 43.968		

Group-no.: 2 (11-20)

645.243	580.901	599.070	719.101	554.762
640.097	608.578	685.897	623.148	603.908
MEDIAN= 615.863	MEAN= 626.071	STD = 48.829		

Group-no.: 3 (21-30)

621.281	634.298	618.577	639.759	583.186
584.019	601.268	642.019	638.791	585.648
MEDIAN= 619.929	MEAN= 614.885	STD = 24.394		

Group-no.: 4 (31-40)

548.596	645.226	559.729	672.984	564.845
655.439	567.982	579.654	634.644	620.683
MEDIAN= 600.169	MEAN= 604.978	STD = 45.662		

BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

CALCULATED F	D.F.s	PROBABILITY
1.4152	3 & 2333.	.2351

HOMOGENEOUS VARIANCES (ONE-TAILED TEST)

ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB
TREATMENT	2244.	3	747.98	.428	.738
ERROR	6.2977E+04	36	1749.4		
TOTAL	6.5221E+04	39			

NO OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL
NO STATISTICAL DIFFERENCE BETWEEN THE GROUPS

[2] Unit of relative lung weights: mg / 100 gram body weight

ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of BODY WEIGHT[3]/FEMALES

```
Group-no.:  1 (1-10)
     417.000      404.000      466.000      448.000      418.000
     441.000      409.000      422.000      418.000      431.000
   MEDIAN=   420.000   MEAN=    427.400    STD =     19.184

Group-no.:  2 (11-20)
     473.000      466.000      430.000      445.000      462.000
     414.000      443.000      390.000      432.000      435.000
   MEDIAN=   439.000   MEAN=    439.000    STD =     25.029

Group-no.:  3 (21-30)
     484.000      449.000      506.000      415.000      452.000
     413.000      473.000      426.000      397.000      432.000
   MEDIAN=   440.500   MEAN=    444.700    STD =     34.718

Group-no.:  4 (31-40)
     463.000      398.000      442.000      496.000      549.000
     478.000      456.000      462.000      407.000      498.000
   MEDIAN=   462.500   MEAN=    464.900    STD =     44.411
```

BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

CALCULATED F	D.F.s	PROBABILITY
2.2108	3 & 2333.	.0835

HOMOGENEOUS VARIANCES (ONE-TAILED TEST)

ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB
TREATMENT	7379.	3	2459.5	2.358	.087
ERROR	3.7549E+04	36	1043.0		
TOTAL	4.4928E+04	39			

NO OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL
NO STATISTICAL DIFFERENCE BETWEEN THE GROUPS

[3] Unit of body weights: gram

Lung Weights - Expos.: 1 x 60 min

```
       ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA
---------------------------------------------------------------------
Analysis of LUNG WEIGHT - absolute/FEMALES
---------------------------------------------------------------------

 Group-no.:  1 (41-50)
    2297.000     2498.000     2563.000     2406.000     2408.000
    2236.000     2266.000     2292.000     2415.000     2744.000
    MEDIAN=  2407.000   MEAN=  2412.500   STD =   156.488

 Group-no.:  2 (51-60)
    2690.000     2872.000     2509.000     3265.000     3008.000
    3365.000     2564.000     2436.000     3049.000     2405.000
    MEDIAN=  2781.000   MEAN=  2816.300   STD =   347.090

 Group-no.:  3 (61-70)
    2611.000     2887.000     2513.000     2468.000     2626.000
    3198.000     2456.000     2496.000     2443.000     2747.000
    MEDIAN=  2562.000   MEAN=  2644.500   STD =   241.240

 Group-no.:  4 (71-80)
    2421.000     2678.000     2593.000     2579.000     3509.000
    2681.000     2193.000     3041.000     2538.000     2957.000
    MEDIAN=  2635.500   MEAN=  2719.000   STD =   368.370
---------------------------------------------------------------------
   BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

  CALCULATED F       D.F.s              PROBABILITY
  ------------    -------------         -----------
     2.2782          3 &   2333.           .0763

   HOMOGENEOUS VARIANCES (ONE-TAILED TEST)
---------------------------------------------------------------------

   ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE
---------------------------------------------------------------------
 SOURCE         SS          DF        MS            F           PROB
---------------------------------------------------------------------
 TREATMENT   8.8838E+05      3     2.96128E+05   3.496         .025
 ERROR       3.0497E+06     36      84713.
 TOTAL       3.9381E+06     39
---------------------------------------------------------------------
   OVERALL SIGNIFICANCE AT  5.% (ONE-TAILED) LEVEL
```

GAMES AND HOWELL MODIFICATION OF
TUKEY-KRAMERs HONESTLY SIGNIFICANT DIFFERENCE TEST
(WITH THE STUDENTIZED RANGE STATISTIC)

GROUPS COMPARED	CALCULATED TEST VALUE	DEGREES OF FREEDOM	PROBABILITY	CONCLUSION
5. % ONE-TAILED TEST				
1 AND 2	4.74	13	.0235	SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 2	4.74	13	.0235	SIGNIFICANT
5. % ONE-TAILED TEST				
1 AND 3	3.61	15	.0918	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 3	3.61	15	.0918	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
1 AND 4	3.42	12	.1255	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 4	3.42	12	.1255	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
2 AND 3	-1.82	16	.5847	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
2 AND 3	1.82	16	.5847	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
2 AND 4	-.86	18	.9282	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
2 AND 4	.86	18	.9282	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
3 AND 4	.76	16	.9492	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
3 AND 4	.76	16	.9492	NOT SIGNIFICANT

ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of LUNG WEIGHT - relative to BODY WEIGHT/FEMALES

Group-no.: 1 (41-50)

557.524	563.883	614.628	606.045	573.333
550.739	579.540	575.879	568.235	672.549

MEDIAN= 574.606 MEAN= 586.236 STD = 36.324

Group-no.: 2 (51-60)

563.941	607.188	571.526	670.431	608.907
624.304	606.147	578.622	639.203	593.827

MEDIAN= 606.667 MEAN= 606.410 STD = 32.407

Group-no.: 3 (61-70)

576.380	611.653	612.927	594.699	557.537
648.682	533.913	588.679	624.808	658.753

MEDIAN= 603.176 MEAN= 600.803 STD = 38.899

Group-no.: 4 (71-80)

597.778	645.301	562.473	564.333	706.036
585.371	568.135	618.089	584.793	638.661

MEDIAN= 591.574 MEAN= 607.097 STD = 45.656

BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

CALCULATED F	D.F.s	PROBABILITY
.3583	3 & 2333.	.7862

HOMOGENEOUS VARIANCES (ONE-TAILED TEST)

ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB
TREATMENT	2815.	3	938.28	.629	.605
ERROR	5.3706E+04	36	1491.8		
TOTAL	5.6521E+04	39			

NO OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL
NO STATISTICAL DIFFERENCE BETWEEN THE GROUPS

ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of BODY WEIGHT/FEMALES

```
Group-no.:  1 (41-50)
     412.000      443.000      417.000      397.000      420.000
     406.000      391.000      398.000      425.000      408.000
   MEDIAN=  410.000   MEAN=   411.700    STD =    15.392

Group-no.:  2 (51-60)
     477.000      473.000      439.000      487.000      494.000
     539.000      423.000      421.000      477.000      405.000
   MEDIAN=  475.000   MEAN=   463.500    STD =    40.970

Group-no.:  3 (61-70)
     453.000      472.000      410.000      415.000      471.000
     493.000      460.000      424.000      391.000      417.000
   MEDIAN=  438.500   MEAN=   440.600    STD =    33.450

Group-no.:  4 (71-80)
     405.000      415.000      461.000      457.000      497.000
     458.000      386.000      492.000      434.000      463.000
   MEDIAN=  457.500   MEAN=   446.800    STD =    36.325
```

BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

CALCULATED F	D.F.s	PROBABILITY
2.5041	3 & 2333.	.0563

HOMOGENEOUS VARIANCES (ONE-TAILED TEST)

ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB
TREATMENT	1.3981E+04	3	4660.2	4.281	.011
ERROR	3.9185E+04	36	1088.5		
TOTAL	5.3165E+04	39			

OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL

GAMES AND HOWELL MODIFICATION OF
TUKEY-KRAMERs HONESTLY SIGNIFICANT DIFFERENCE TEST
(WITH THE STUDENTIZED RANGE STATISTIC)

GROUPS COMPARED	CALCULATED TEST VALUE	DEGREES OF FREEDOM	PROBABILITY	CONCLUSION
5. % ONE-TAILED TEST				
1 AND 2	5.29	11	.0147	SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 2	5.29	11	.0147	SIGNIFICANT
5. % ONE-TAILED TEST				
1 AND 3	3.51	13	.1100	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 3	3.51	13	.1100	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
1 AND 4	3.98	12	.0653	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 4	3.98	12	.0653	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
2 AND 3	-1.94	17	.5343	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
2 AND 3	1.94	17	.5343	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
2 AND 4	-1.36	18	.7709	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
2, AND 4	1.36	18	.7709	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
3 AND 4	.56	18	.9781	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
3 AND 4	.56	18	.9781	NOT SIGNIFICANT

Lung Weights - Expos.: 1 x 6 hours

ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of LUNG WEIGHT - absolute/FEMALES

```
Group-no.:  1 (81-90)
    2763.000     2490.000     2408.000     2604.000     2261.000
    2490.000     2700.000     2744.000     2858.000     2679.000
    MEDIAN=  2641.500    MEAN=  2599.700    STD =   184.424

Group-no.:  2 (91-100)
    3004.000     3004.000     2602.000     2897.000     2960.000
    2883.000     2610.000     2962.000     2387.000     3046.000
    MEDIAN=  2928.500    MEAN=  2835.500    STD =   222.401

Group-no.:  3 (101-110)
    2657.000     2765.000     2282.000     2316.000     2922.000
    2380.000     2602.000     2201.000     2463.000     2693.000
    MEDIAN=  2532.500    MEAN=  2528.100    STD =   235.529

Group-no.:  4 (111-120)
    2394.000     2583.000     2574.000     2451.000     2645.000
    2426.000     2864.000     2975.000     2854.000     2558.000
    MEDIAN=  2578.500    MEAN=  2632.400    STD =   201.110
```

BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

CALCULATED F	D.F.s	PROBABILITY
.1987	3 & 2333.	.8973

HOMOGENEOUS VARIANCES (ONE-TAILED TEST)

ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB
TREATMENT	5.2105E+05	3	1.73684E+05	3.873	.017
ERROR	1.6145E+06	36	44848.		
TOTAL	2.1356E+06	39			

OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL

GAMES AND HOWELL MODIFICATION OF
TUKEY-KRAMERs HONESTLY SIGNIFICANT DIFFERENCE TEST
(WITH THE STUDENTIZED RANGE STATISTIC)

GROUPS COMPARED	CALCULATED TEST VALUE	DEGREES OF FREEDOM	PROBABILITY	CONCLUSION
5. % ONE-TAILED TEST				
1 AND 2	3.65	17	.0825	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 2	3.65	17	.0825	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
1 AND 3	-1.07	17	.8725	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 3	1.07	17	.8725	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
1 AND 4	.54	18	.9809	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 4	.54	18	.9809	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
2 AND 3	-4.24	18	.0352	SIGNIFICANT
5. % TWO-TAILED TEST				
2 AND 3	4.24	18	.0352	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
2 AND 4	-3.03	18	.1778	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
2 AND 4	3.03	18	.1778	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
3 AND 4	1.51	18	.7144	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
3 AND 4	1.51	18	.7144	NOT SIGNIFICANT

ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of LUNG WEIGHT - relative to BODY WEIGHT/FEMALES

Group-no.: 1 (81-90)

683.911	591.449	620.619	644.554	621.154
631.980	665.025	604.405	677.251	618.707

MEDIAN= 626.567 MEAN= 635.906 STD = 31.034

Group-no.: 2 (91-100)

616.838	628.452	619.524	639.514	614.108
601.879	568.627	643.913	536.404	620.367

MEDIAN= 618.181 MEAN= 608.963 STD = 33.010

Group-no.: 3 (101-110)

601.131	666.265	606.915	555.396	627.039
593.516	647.264	543.457	530.819	546.247

MEDIAN= 597.323 MEAN= 591.805 STD = 46.711

Group-no.: 4 (111-120)

570.000	615.000	637.129	576.706	634.293
550.113	666.047	645.336	638.479	623.902

MEDIAN= 629.098 MEAN= 615.701 STD = 37.587

BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

CALCULATED F	D.F.s	PROBABILITY
.5893	3 & 2333.	.6263

HOMOGENEOUS VARIANCES (ONE-TAILED TEST)

ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB
TREATMENT	9975.	3	3324.8	2.355	.087
ERROR	5.0828E+04	36	1411.9		
TOTAL	6.0802E+04	39			

NO OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL
NO STATISTICAL DIFFERENCE BETWEEN THE GROUPS

```
          ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA
-----------------------------------------------------------------
Analysis of BODY WEIGHT/FEMALES
-----------------------------------------------------------------

 Group-no.:  1 (81-90)
      404.000      421.000      388.000      404.000      364.000
      394.000      406.000      454.000      422.000      433.000
    MEDIAN=   405.000   MEAN=   409.000    STD =     25.087

 Group-no.:  2 (91-100)
      487.000      478.000      420.000      453.000      482.000
      479.000      459.000      460.000      445.000      491.000
    MEDIAN=   469.000   MEAN=   463.400    STD =     22.217

 Group-no.:  3 (101-110)
      442.000      415.000      37[illegible].000      417.000      466.000
      401.000      402.000      405.000      464.000      493.000
    MEDIAN=   416.000   MEAN=   428.100    STD =     36.650

 Group-no.:  4 (111-120)
      420.000      420.000      404.000      425.000      417.000
      441.000      430.000      461.000      447.000      410.000
    MEDIAN=   422.500   MEAN=   427.500    STD =     17.570
-----------------------------------------------------------------
   BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

  CALCULATED F        D.F.s              PROBABILITY
  ------------   ---------------        ------------
     1.6835          3 &   2333.           .1668

   HOMOGENEOUS VARIANCES (ONE-TAILED TEST)
-----------------------------------------------------------------

   ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE
-----------------------------------------------------------------
 SOURCE          SS            DF         MS           F         PROB
-----------------------------------------------------------------
 TREATMENT   1.6790E+04        3        5596.7       8.068       .001
 ERROR       2.4974E+04       36        693.72
 TOTAL       4.1764E+04       39
-----------------------------------------------------------------
   OVERALL SIGNIFICANCE AT  5.% (ONE-TAILED) LEVEL
```

GAMES AND HOWELL MODIFICATION OF
TUKEY-KRAMERs HONESTLY SIGNIFICANT DIFFERENCE TEST
(WITH THE STUDENTIZED RANGE STATISTIC)

GROUPS COMPARED	CALCULATED TEST VALUE	DEGREES OF FREEDOM	PROBABILITY	CONCLUSION
5. % ONE-TAILED TEST				
1 AND 2	7.53	18	.0002	SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 2	7.53	18	.0002	SIGNIFICANT
5. % ONE-TAILED TEST				
1 AND 3	1.92	16	.5406	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 3	1.92	16	.5406	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
1 AND 4	2.70	16	.2628	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 4	2.70	16	.2628	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
2 AND 3	-3.89	15	.0637	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
2 AND 3	3.89	15	.0637	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
2 AND 4	-5.98	17	.0029	SIGNIFICANT
5. % TWO-TAILED TEST				
2 AND 4	5.98	17	.0029	SIGNIFICANT
5. % ONE-TAILED TEST				
3 AND 4	-.07	13	1.0000	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
3 AND 4	.07	13	1.0000	NOT SIGNIFICANT

Gross necropsy

Individual findings / female guinea pigs (15 min Exposure)

Group	Animal No.	Time of death	Sacrificed after	Pathology findings
1 (1x15 min)	1		22 d	no observable findings
	2		22 d	no observable findings
	3		22 d	no observable findings
	4		22 d	**liver**: white focus (∅≈ 3mm)
	5		22 d	no observable findings
	6		22 d	no observable findings
	7		22 d	no observable findings
	8		22 d	no observable findings
	9		22 d	no observable findings
	10		22 d	no observable findings
2 (1x15 min)	11		22 d	no observable findings
	12		22 d	no observable findings
	13		22 d	no observable findings
	14		22 d	**lung**: dark red foci (∅≈ 1 mm)
	15		22 d	**lung**: dark red foci (∅≈ 1 mm)
	16		22 d	no observable findings
	17		22 d	no observable findings
	18		22 d	no observable findings
	19		22 d	no observable findings
	20		22 d	**lung**: dark red focus (∅≈ 2 mm)
3 (1x15 min)	21		22 d	no observable findings
	22		22 d	no observable findings
	23		22 d	no observable findings
	24		22 d	no observable findings
	25		22 d	no observable findings
	26		22 d	no observable findings
	27		22 d	no observable findings
	28		22 d	no observable findings
	29		22 d	no observable findings
	30		22 d	no observable findings
4 (1x15 min)	31		22 d	no observable findings
	32		22 d	no observable findings
	33		22 d	no observable findings
	34		22 d	no observable findings
	35		22 d	no observable findings
	36		22 d	no observable findings
	37		22 d	no observable findings
	38		22 d	no observable findings
	39		22 d	no observable findings
	40		22 d	no observable findings

Individual findings / female guinea pigs (1 h Exposure)

Group	Animal No.	Time of death	Sacrificed after	Pathology findings
5 (1x1 hr)	41		23 d	no observable findings
	42		23 d	no observable findings
	43		23 d	no observable findings
	44		23 d	**lung**: dark red foci (∅≈ 1mm)
	45		23 d	no observable findings
	46		23 d	no observable findings
	47		23 d	no observable findings
	48		23 d	no observable findings
	49		23 d	no observable findings
	50		23 d	no observable findings
6 (1x1 hr)	51		23 d	no observable findings
	52		23 d	no observable findings
	53		23 d	no observable findings
	54		23 d	**lung**: dark red foci (∅≈ 1mm)
	55		23 d	no observable findings
	56		23 d	no observable findings
	57		23 d	no observable findings
	58		23 d	no observable findings
	59		23 d	no observable findings
	60		23 d	no observable findings
7 (1x1 hr)	61		23 d	no observable findings
	62		23 d	no observable findings
	63		23 d	no observable findings
	64		23 d	no observable findings
	65		23 d	**liver**: white focus (∅≈ 3mm)
	66		23 d	no observable findings
	67		23 d	no observable findings
	68		23 d	no observable findings
	69		23 d	no observable findings
	70		23 d	**lung**: dark red foci (∅≈ 1mm)
8 (1x1 hr)	71		23 d	no observable findings
	72		23 d	no observable findings
	73		23 d	no observable findings
	74		23 d	no observable findings
	75		23 d	no observable findings
	76		23 d	no observable findings
	77		23 d	no observable findings
	78		23 d	**liver**:several white foci
	79		23 d	no observable findings
	80		23 d	no observable findings

Individual findings / female guinea pigs (6 h Exposure)

Group	Animal No.	Time of death	Sacrificed after	Pathology findings
9 (1x6 hr)	81		22 d	**lung**: gray focus (Ø≈ 1 mm)
	82		22 d	no observable findings
	83		22 d	no observable findings
	84		22 d	no observable findings
	85		22 d	no observable findings
	86		22 d	no observable findings
	87		22 d	no observable findings
	88		22 d	no observable findings
	89		22 d	no observable findings
	90		22 d	no observable findings
10 (1x6 hr)	91		22 d	no observable findings
	92		22 d	no observable findings
	93		22 d	no observable findings
	94		22 d	no observable findings
	95		22 d	no observable findings
	96		22 d	**liver**: several white foci (Ø≈ 2 mm)
	97		22 d	no observable findings
	98		22 d	no observable findings
	99		22 d	**liver**: several white foci (Ø≈ 2 mm)
	100		22 d	no observable findings
11 (1x6 hr)	101		22 d	no observable findings
	102		22 d	no observable findings
	103		22 d	no observable findings
	104		22 d	no observable findings
	105		22 d	no observable findings
	106		22 d	no observable findings
	107		22 d	no observable findings
	108		22 d	no observable findings
	109		22 d	no observable findings
	110		22 d	no observable findings
12 (1x6 hr)	111		22 d	no observable findings
	112		22 d	no observable findings
	113		22 d	no observable findings
	114		22 d	no observable findings
	115		22 d	no observable findings
	116		22 d	no observable findings
	117		22 d	no observable findings
	118		22 d	no observable findings
	119		22 d	no observable findings
	120		22 d	no observable findings

Incidence Table - Macroscopic Lung Findings

Macroscopic lung findings

R X C CHI-SQUARE - TEST:

```
Chisquare =    16.233  DF = 11  Frequency =      .583
Chi-Tab. =     19.675  p = 0.05 (bilateral)
```

FISHERs EXACT TEST:

Group: 1		Incidence:	0/10	B	U
Group: 2	P= .1053	Incidence:	3/10	-	-
Group: 3	P= 1.0000	Incidence:	0/10	-	-
Group: 4	P= 1.0000	Incidence:	0/10	-	-
Group: 5	P= .5000	Incidence:	1/10	-	-
Group: 6	P= .5000	Incidence:	1/10	-	-
Group: 7	P= .5000	Incidence:	1/10	-	-
Group: 8	P= 1.0000	Incidence:	0/10	-	-
Group: 9	P= .5000	Incidence:	1/10	-	-
Group:10	P= 1.0000	Incidence:	0/10	-	-
Group:11	P= 1.0000	Incidence:	0/10	-	-
Group:12	P= 1.0000	Incidence:	0/10	-	-

B = bilateral comparison of groups
U = unilateral comparison of groups
P = single-tailed probability

#/# = 1st figure: number of positive observations
2nd figure: number of total observations

B 05

13. APPENDIX - REPEATED EXPOSURE

Scheduling / Calendar

Desmodur 44 V 20 L
T7062289

S C H E D U L I N G   C A L E N D A R   page: 1

Targeted date for pre-study examinations:
Targeted date for start of study: 26.01.1998
Actual date for start of study: 26.01.1998
Time Scale: A
Offset (in days): 0

Day	Date [dd.mm.yy]	pre. exam.	Calendar rel. Day	Calendar rel. Week	Calendar abs. Day	Calendar abs. Week
Mo	26.01.98		0	0	0	0
Tu	27.01.98		1	0	1	0
We	28.01.98		2	0	2	0
Th	29.01.98		3	0	3	0
Fr	30.01.98		4	0	4	0
Sa	31.01.98		5	0	5	0
Su	01.02.98		6	0	6	0
Mo	02.02.98		7	1	7	1
Tu	03.02.98		8	1	8	1
We	04.02.98		9	1	9	1
Th	05.02.98		10	1	10	1
Fr	06.02.98		11	1	11	1
Sa	07.02.98		12	1	12	1
Su	08.02.98		13	1	13	1
Mo	09.02.98		14	2	14	2
Tu	10.02.98		15	2	15	2
We	11.02.98		16	2	16	2
Th	12.02.98		17	2	17	2
Fr	13.02.98		18	2	18	2
Sa	14.02.98		19	2	19	2
Su	15.02.98		20	2	20	2
Mo	16.02.98		21	3	21	3
Tu	17.02.98		22	3	22	3
We	18.02.98		23	3	23	3
Th	19.02.98		24	3	24	3
Fr	20.02.98		25	3	25	3
Sa	21.02.98		26	3	26	3
Su	22.02.98		27	3	27	3
Mo	23.02.98		28	4	28	4
Tu	24.02.98		29	4	29	4
We	25.02.98		30	4	30	4
Th	26.02.98		31	4	31	4
Fr	27.02.98		32	4	32	4
Sa	28.02.98		33	4	33	4
Su	01.03.98		34	4	34	4
Mo	02.03.98		35	5	35	5
Tu	03.03.98		36	5	36	5
We	04.03.98		37	5	37	5

Desmodur 44 V 20 L
T7062289

S C H E D U L I N G   C A L E N D A R   page: 2

Day	Date [dd.mm.yy]	pre. exam.	Calendar rel. Day	Calendar rel. Week	Calendar abs. Day	Calendar abs. Week
Th	05.03.98		38	5	38	5
Fr	06.03.98		39	5	39	5
Sa	07.03.98		40	5	40	5
Su	08.03.98		41	5	41	5
Mo	09.03.98		42	6	42	6
Tu	10.03.98		43	6	43	6
We	11.03.98		44	6	44	6
Th	12.03.98		45	6	45	6
Fr	13.03.98		46	6	46	6
Sa	14.03.98		47	6	47	6
Su	15.03.98		48	6	48	6
Mo	16.03.98		49	7	49	7
Tu	17.03.98		50	7	50	7
We	18.03.98		51	7	51	7
Th	19.03.98		52	7	52	7
Fr	20.03.98		53	7	53	7

Analytical concentrations/test atmosphere (Nitroreagent)

		Target concentration - mg/m³ air			
Date	Day	0	1	3	10
02.03.98	0	--	.961	2.270	10.420
02.03.98	0	--	1.070	2.620	10.460
02.03.98	0	--	.969	2.300	11.510
03.03.98	1	--	1.090	2.690	9.860
03.03.98	1	--	.971	2.750	11.180
03.03.98	1	--	1.080	2.910	9.410
04.03.98	2	--	1.170	2.570	23.590
04.03.98	2	--	1.050	2.810	12.090
04.03.98	2	--	1.210	3.040	12.550
05.03.98	3	--	.790	2.790	8.280
05.03.98	3	--	1.170	4.030	9.580
05.03.98	3	--	.919	3.910	8.840
06.03.98	4	--	.894	3.270	11.760
06.03.98	4	--	1.050	2.840	13.240
06.03.98	4	--	1.230	2.650	13.320
09.03.98	7	--	.925	2.470	4.840
09.03.98	7	--	.880	2.370	11.320
09.03.98	7	--	.940	2.360	11.030
10.03.98	8	--	.833	2.440	12.040
10.03.98	8	--	.838	2.830	11.840
10.03.98	8	--	.824	2.810	10.270
10.03.98	8	--	--	--	11.780
11.03.98	9	--	.856	3.980	12.590
11.03.98	9	--	1.000	3.250	12.740
11.03.98	9	--	1.040	3.450	12.000
11.03.98	9	--	--	--	13.320
12.03.98	10	--	1.180	3.060	--
12.03.98	10	--	1.250	3.170	--
12.03.98	10	--	1.200	3.010	--
12.03.98	10	--	--	--	--
13.03.98	11	--	1.400	3.760	14.460
13.03.98	11	--	1.500	4.320	12.880
13.03.98	11	--	1.470	3.690	12.190
13.03.98	11	--	--	--	--
15.03.98	13	--	--	--	--
15.03.98	13	--	1.110	2.740	14.820
15.03.98	13	--	1.450	1.960	10.050
15.03.98	13	--	1.170	2.390	11.020
16.03.98	14	--	1.200	3.310	14.360
16.03.98	14	--	1.110	3.600	16.290
16.03.98	14	--	1.490	3.690	16.070
16.03.98	14	--	--	--	--
17.03.98	15	--	1.230	2.410	9.090
17.03.98	15	--	1.710	2.620	12.100
17.03.98	15	--	1.200	2.450	10.550
17.03.98	15	--	--	--	--

Analytical concentrations/test atmosphere

		Target concentration - mg/m³ air			
Date	Day	0	1	3	10
18.03.98	16	--	1.060	2.680	10.410
18.03.98	16	--	1.060	2.440	12.040
18.03.98	16	--	.900	2.110	10.270
18.03.98	16	--	--	--	--
19.03.98	17	--	1.590	3.370	13.420
19.03.98	17	--	1.600	3.450	12.920
19.03.98	17	--	1.370	3.440	12.540
19.03.98	17	--	--	--	--
MEAN			1.133	2.956	11.939
STD			.230	.567	2.737

Dimension of data: mg/m³ air
-- = not measured or not evaluated

Analytical concentrations/test atmosphere (Filter)

		Target concentration - mg/m³ air			
Date	Day	0	1	3	10
02.03.98	0	--	1.275	3.560	13.810
03.03.98	1	--	1.280	3.410	9.030
04.03.98	2	--	1.560	3.355	13.540
05.03.98	3	--	1.065	5.250	9.230
06.03.98	4	--	1.195	3.600	12.800
09.03.98	7	--	1.015	3.240	7.770
10.03.98	8	--	1.100	3.100	7.130
11.03.98	9	--	1.400	4.665	14.940
12.03.98	10	--	1.550	3.940	17.820
13.03.98	11	--	1.780	4.485	11.980
13.03.98	11	--	--	--	13.770
15.03.98	13	--	1.440	3.510	10.760
15.03.98	13	--	--	--	8.950
15.03.98	13	--	--	--	12.280
16.03.98	14	--	1.970	4.685	20.620
16.03.98	14	--	--	3.655	17.020
17.03.98	15	--	1.200	3.080	10.070
18.03.98	16	--	1.310	2.295	10.610
18.03.98	16	--	--	2.8[illegible]0	12.040
19.03.98	17	--	1.455	3.720	14.020
MEAN			1.373	3.668	12.409
STD			.264	.747	3.455

Dimension of data: mg/m³ air
-- = not measured or not evaluated

Temperature / test atmosphere

		Target concentration - mg/m³ air			
Date	Day	0	1	3	10
02.03.98	0	22.900	22.500	22.300	22.600
03.03.98	1	23.300	22.700	22.600	23.000
04.03.98	2	23.200	23.000	22.700	23.400
05.03.98	3	23.200	22.700	22.600	23.000
06.03.98	4	23.400	22.800	22.700	23.100
09.03.98	7	23.300	22.800	22.700	23.000
10.03.98	8	23.300	22.800	22.700	23.000
11.03.98	9	23.500	22.800	22.900	23.100
12.03.98	10	23.600	22.900	23.100	23.400
13.03.98	11	23.600	23.000	23.000	23.400
15.03.98	13	23.600	23.000	22.900	23.200
16.03.98	14	23.500	23.000	23.100	23.300
17.03.98	15	23.400	23.000	22.800	23.200
18.03.98	16	23.300	23.000	22.900	23.200
19.03.98	17	23.300	23.000	23.000	23.400
MEAN		23.360	22.866	22.800	23.153
STD		.188	.154	.217	.219

Dimension of data: Deg. Cel.

Relative humidity / test atmosphere

Date	Day	Target concentration - mg/m³ air 0	1	3	10
02.03.98	0	9.140	9.230	7.840	12.940
03.03.98	1	1.990	1.830	.110	6.590
04.03.98	2	1.990	1.780	.180	6.040
05.03.98	3	2.000	1.810	.100	6.050
06.03.98	4	2.070	1.860	.160	6.130
09.03.98	7	2.010	1.710	.300E-01	5.950
10.03.98	8	2.080	1.830	.120	6.100
11.03.98	9	1.960	1.750	.833E-02	5.990
12.03.98	10	1.940	1.730	.300E-01	5.960
13.03.98	11	2.370	2.150	.200E-01	6.480
15.03.98	13	1.940	1.740	.400E-01	6.070
16.03.98	14	2.070	1.790	.800E-01	6.310
17.03.98	15	1.980	1.780	.400E-01	6.110
18.03.98	16	1.970	1.790	.700E-01	6.140
19.03.98	17	1.960	1.780	.700E-01	6.150
MEAN		2.498	2.304	.593	6.600
STD		1.840	1.918	2.005	1.763

Dimension of data: %

Particle analysis / test atmosphere

Target concentration - mg/m³ air : 1

Date	Day	NMAD	MMAD	GSD	Resp.	Recov.
03.03.98	1	.740	1.600	1.660	89.300	1.290
04.03.98	2	.250	1.170	2.040	90.700	1.460
06.03.98	4	.690	1.470	1.650	92.300	1.150
09.03.98	7	.830	1.410	1.520	96.500	1.020
10.03.98	8	.850	1.430	1.520	96.100	.990
12.03.98	10	.770	1.460	1.590	94.100	1.310
15.03.98	13	.780	1.510	1.600	92.700	1.360
16.03.98	14	.780	1.490	1.590	93.300	1.320
18.03.98	16	.650	1.420	1.670	92.700	.900
MEAN		.704	1.440	1.648	93.077	1.200
STD		.181	.116	.156	2.311	.192

NMAD: number median aerodynamic diameter - ~m
MMAD: mass median aerodynamic diameter - ~m
GSD: geometric standard deviation
Resp.: respirability, i.e. relative particle mass ^a<^n 3 ~m (%)
Recov.: mg/m³ air (impactor)

Particle analysis / test atmosphere

Target concentration - mg/m³ air : 3

Date	Day	NMAD	MMAD	GSD	Resp.	Recov.
02.03.98	0	.710	1.420	1.620	93.90	3.420
04.03.98	2	.370	1.610	2.010	81.50	2.970
05.03.98	3	.690	1.410	1.630	93.90	4.570
09.03.98	7	.590	1.330	1.680	94.10	2.640
11.03.98	9	.680	1.430	1.640	93.10	4.110
13.03.98	11	.730	1.410	1.590	94.90	3.740
15.03.98	13	.710	1.460	1.630	92.90	3.470
17.03.98	15	.720	1.490	1.640	92.10	3.280
19.03.98	17	.830	1.490	1.560	94.30	3.260
MEAN		.670	1.450	1.666	92.30	3.495
STD		.128	.772E-01	.133	4.13	.581

NMAD: number median aerodynaric diameter - ~m
MMAD: mass median aerodynamic diameter - ~m
GSD: geometric standard deviation
Resp.: respirability, i.e. relative particle mass ^a<^n 3 ~m (%)
Recov.: mg/m³ air (impactor)

Particle analysis / test atmosphere

Target concentration - mg/m³ air : 10

Date	Day	NMAD	MMAD	GSD	Resp.	Recov.
03.03.98	1	.740	1.690	1.690	86.500	10.910
04.03.98	2	.760	1.470	1.600	93.500	15.680
06.03.98	4	.860	1.540	1.560	93.500	13.690
09.03.98	7	.840	1.540	1.570	93.100	8.730
11.03.98	9	.810	1.570	1.600	91.500	15.280
13.03.98	11	.820	1.610	1.610	90.300	14.630
15.03.98	13	.800	1.600	1.620	90.500	12.960
17.03.98	15	.830	1.620	1.600	90.500	10.580
19.03.98	17	.750	1.510	1.620	92.300	14.240
MEAN		.801	1.572	1.607	91.300	12.966
STD		.422E-01	.659E-01	.370E-01	2.215	2.384

NMAD: number median aerodynamic diameter - ~m
MMAD: mass median aerodynamic diameter - ~m
GSD: geometric standard deviation
Resp.: respirability, i.e. relative particle mass ^a<^n 3 ~m (%)
Recov.: mg/m³ air (impactor)

Characterization of Particle Size Distribution (Examples)

ANALYSIS OF PARTICLE DISTRIBUTIONS

Type of investigation: Acute Inhalation - Aerosol

Compound: PMDI

Date of exposure: 06.03.1998 Study-no.: T7062289
Nominal concentration: 1.0 mg/m3 air

N	Impactor stage (um - um)	Cut-Off diameter (um)	Mass/ stage (mg)	Rel. mass (%)	Cumul. mass (%)
1	.06 - .12	.060	.000	.00	.00
2	.12 - .25	.120	.002	.26	.00
3	.25 - .49	.250	.011	1.43	.26
4	.49 - .90	.490	.101	13.13	1.69
5	.90 - 1.85	.900	.403	52.41	14.82
6	1.85 - 3.69	1.850	.228	29.65	67.23
7	3.69 - 7.42	3.690	.021	2.73	96.88
8	7.42 -14.80	7.420	.003	.39	99.61
9	14.80 -30.00	14.800	.000	.00	100.00

Mass Median Aerodynamic Diameter (MMAD): 1.47 um
Geometric standard deviation (GSD): 1.65
Number Median Aerodynamic Diameter (NMAD): .69 um
Surface Median Aerodynamic Diameter (SMAD): 1.14 um

System: BERNER-IMPACTOR I
Air flow: 5.56 liter/min.
Sampling time: 7200.00 seconds
Concentration (computed): 1.15 mg/m3 air

Respirability (percent < 1.0 um):

1. Mass related: 22.5 % (measured)
2. Number related: 76.9 % (extrapolated)

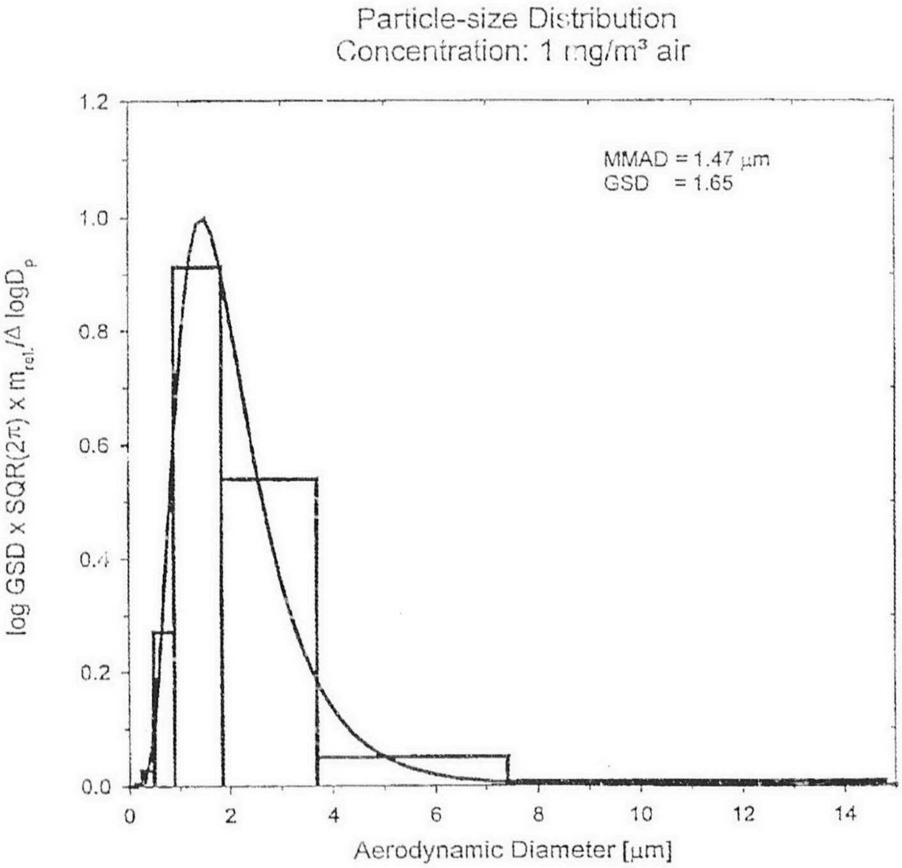
Respirability (percent < 3.0 um):

1. Mass relate(92.3 % (measured)
2. Number related: 99.1 % (extrapolated)

Respirability (percent < 5.0 um):

1. Mass related: 99.1 % (measured)
2. Number related: 99.1 % (extrapolated)

ECD-definition: right cut-size (Dp+1)


Particle-size Distribution
Concentration: 1 mg/m³ air
MMAD = 1.47 µm
GSD = 1.65
log GSD x SQR(2π) x m_rel./Δ logD_p
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
14
Aerodynamic Diameter [µm]

ANALYSIS OF PARTICLE DISTRIBUTIONS

Type of investigation: Acute Inhalation - Aerosol

Compound: PMDI

Date of exposure: 11.03.1998 Study-no.: T7062289
Nominal concentration: 3.0 mg/m3 air

N	Impactor stage (um - um)	Cut-Off diameter (um)	Mass/ stage (mg)	Rel. mass (%)	Cumul. mass (%)
1	.06 - .12	.060	.001	.07	.00
2	.12 - .25	.120	.006	.44	.07
3	.25 - .49	.250	.019	1.38	.51
4	.49 - .90	.490	.182	13.27	1.90
5	.90 - 1.85	.900	.755	55.03	15.16
6	1.85 - 3.69	1.850	.371	27.04	70.19
7	3.69 - 7.42	3.690	.031	2.26	97.23
8	7.42 -14.80	7.420	.007	.51	99.49
9	14.80 -30.00	14.800	.000	.00	100.00

Mass Median Aerodynamic Diameter (MMAD): 1.43 um
Geometric standard deviation (GSD): 1.64
Number Median Aerodynamic Diameter (NMAD): .68 um
Surface Median Aerodynamic Diameter (SMAD): 1.12 um

System: BERNER-IMPACTOR I
Air flow: 5.56 liter/min.
Sampling time: 3600.00 seconds
Concentration (computed): 4.11 mg/m3 air

Respirability (percent < 1.0 um):

1. Mass related: 23.7 % (measured)
2. Number related: 77.9 % (extrapolated)

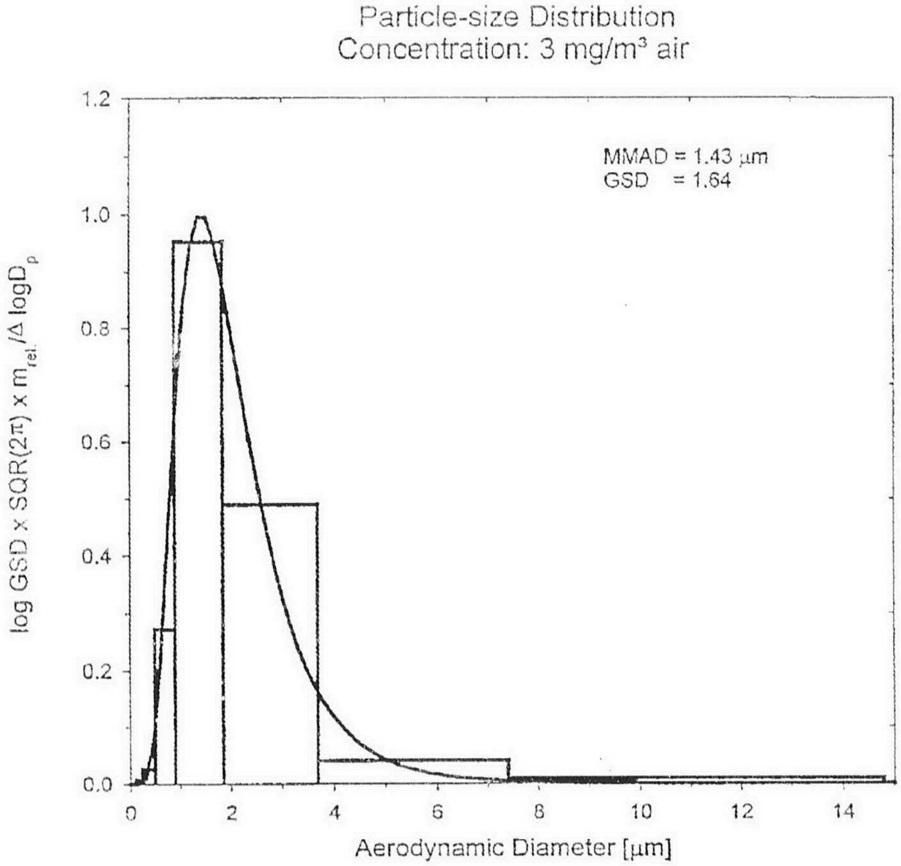
Respirability (percent < 3.0 um):

1. Mass related: 93.1 % (measured)
2. Number related: 99.1 % (extrapolated)

Respirability (percent < 5.0 um):

1. Mass related: 99.1 % (measured)
2. Number related: 99.1 % (extrapolated)

ECD-definition: right cut-size (Dp+1)


Particle-size Distribution
Concentration: 3 mg/m³ air
MMAD = 1.43 µm
GSD = 1.64
log GSD x SQR(2π) x m_rel./Δ logD_p
Aerodynamic Diameter [µm]
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0
2
4
6
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C 05

ANALYSIS OF PARTICLE DISTRIBUTIONS

Type of investigation: Acute Inhalation - Aerosol

Compound: PMDI

Date of exposure: 13.03.1998 Study-no.: T7062289
Nominal concentration: 10.0 mg/m3 air

N	Impactor stage (um - um)	Cut-Off diameter (um)	Mass/ stage (mg)	Rel. mass (%)	Cumul. mass (%)
1	.06 - .12	.060	.000	.00	.00
2	.12 - .25	.120	.000	.00	.00
3	.25 - .49	.250	.012	.49	.00
4	.49 - .90	.490	.256	10.49	.49
5	.90 - 1.85	.900	1.250	51.21	10.98
6	1.85 - 3.69	1.850	.818	33.51	62.19
7	3.69 - 7.42	3.690	.102	4.18	95.70
8	7.42 -14.80	7.420	.003	.12	99.88
9	14.80 -30.00	14.800	.000	.00	100.00

Mass Median Aerodynamic Diameter (MMAD): 1.61 um
Geometric standard deviation (GSD): 1.61
Number Median Aerodynamic Diameter (NMAD): .82 um
Surface Median Aerodynamic Diameter (SMAD): 1.28 um

System: BERNER-IMPACTOR I
Air flow: 5.56 liter/min.
Sampling time: 1800.00 seconds
Concentration (computed): 14.63 mg/m3 air

Respirability (percent < 1.0 um):

1. Mass related: 16.1 % (measured)
2. Number related: 66.5 % (extrapolated)

Respirability (percent < 3.0 um):

1. Mass related: 90.3 % (measured)
2. Number related: 99.1 % (extrapolated)

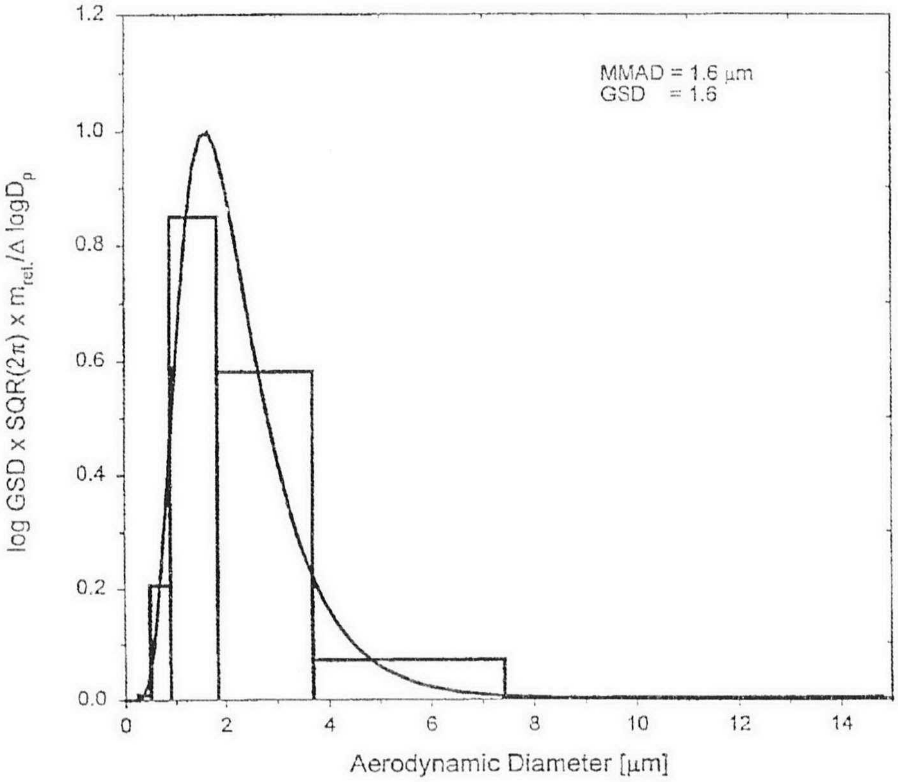
Respirability (percent < 5.0 um):

1. Mass related: 99.1 % (measured)
2. Number related: 99.1 % (extrapolated)

ECD-definition: right cut-size (Dp+1)

C 06

Particle-size Distribution
Concentration: 10 mg/m³ air



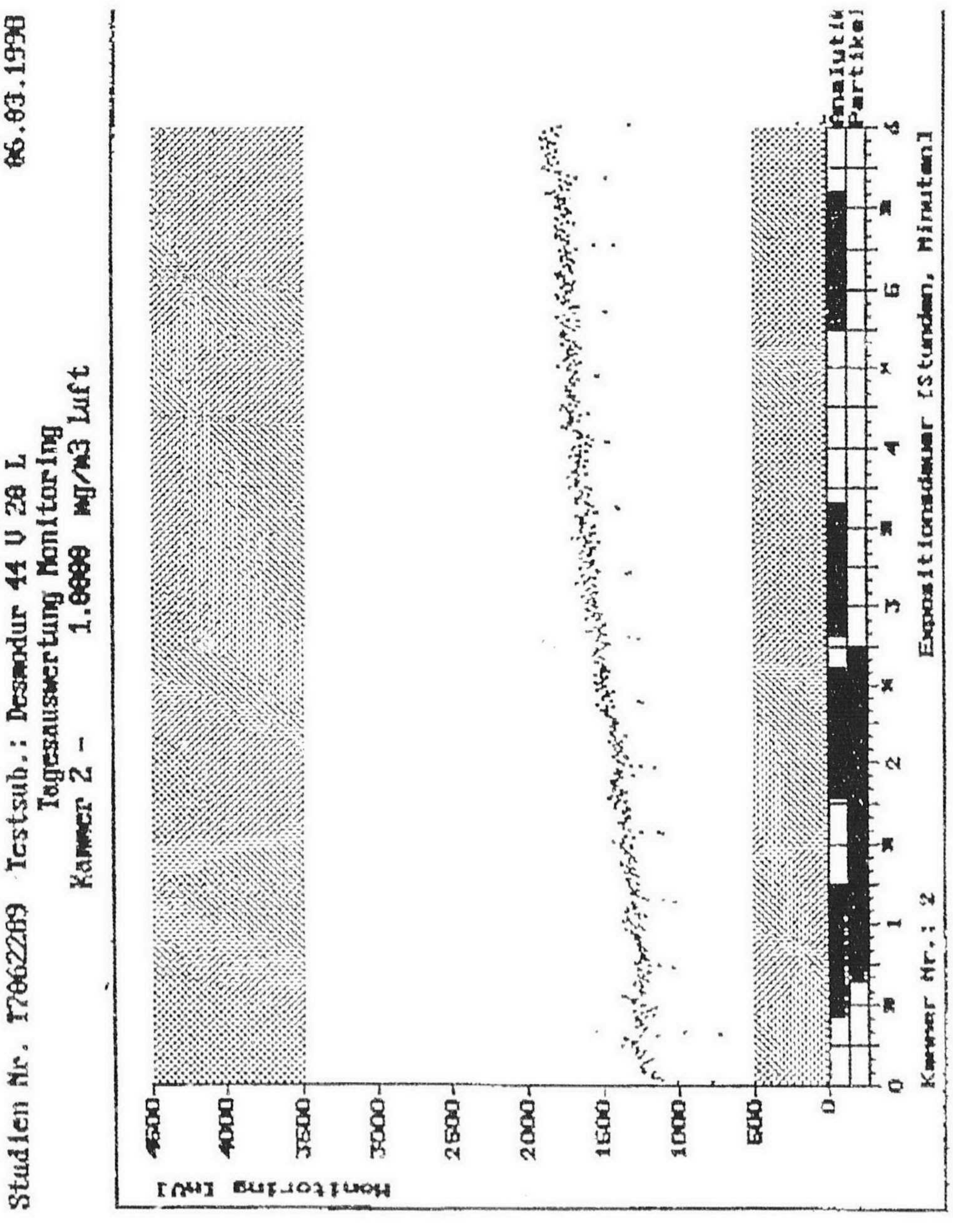
Monitoring / test atmosphere

		Target concentration - mg/m³ air			
Date	Day	0	1	3	10
02.03.98	0	--	1780.0	4994.0	1895.0
03.03.98	1	--	1717.0	4667.0	1572.0
04.03.98	2	--	1661.0	4439.0	2715.0
05.03.98	3	--	1251.0	5015.0	1014.0
06.03.98	4	--	1521.0	3880.0	1902.0
09.03.98	7	--	1333.0	3746.0	1677.0
10.03.98	8	--	1230.0	3876.0	1876.0
11.03.98	9	--	1724.0	5215.0	2912.0
12.03.98	10	--	1805.0	4900.0	2363.0
13.03.98	11	--	1759.0	5201.0	2571.0
15.03.98	13	--	1906.0	4635.0	1916.0
16.03.98	14	--	1557.0	5215.0	3160.0
17.03.98	15	--	1545.0	4370.0	2060.0
18.03.98	16	--	1011.0	3451.0	1871.0
19.03.98	17	--	1865.0	4636.0	3029.0
MEAN			1577.6	4549.3	2168.8
STD			264.1	577.8	603.5

Dimension of data: A.U.
-- = not measured or not evaluated

Monitoring of Atmosphere (Examples)

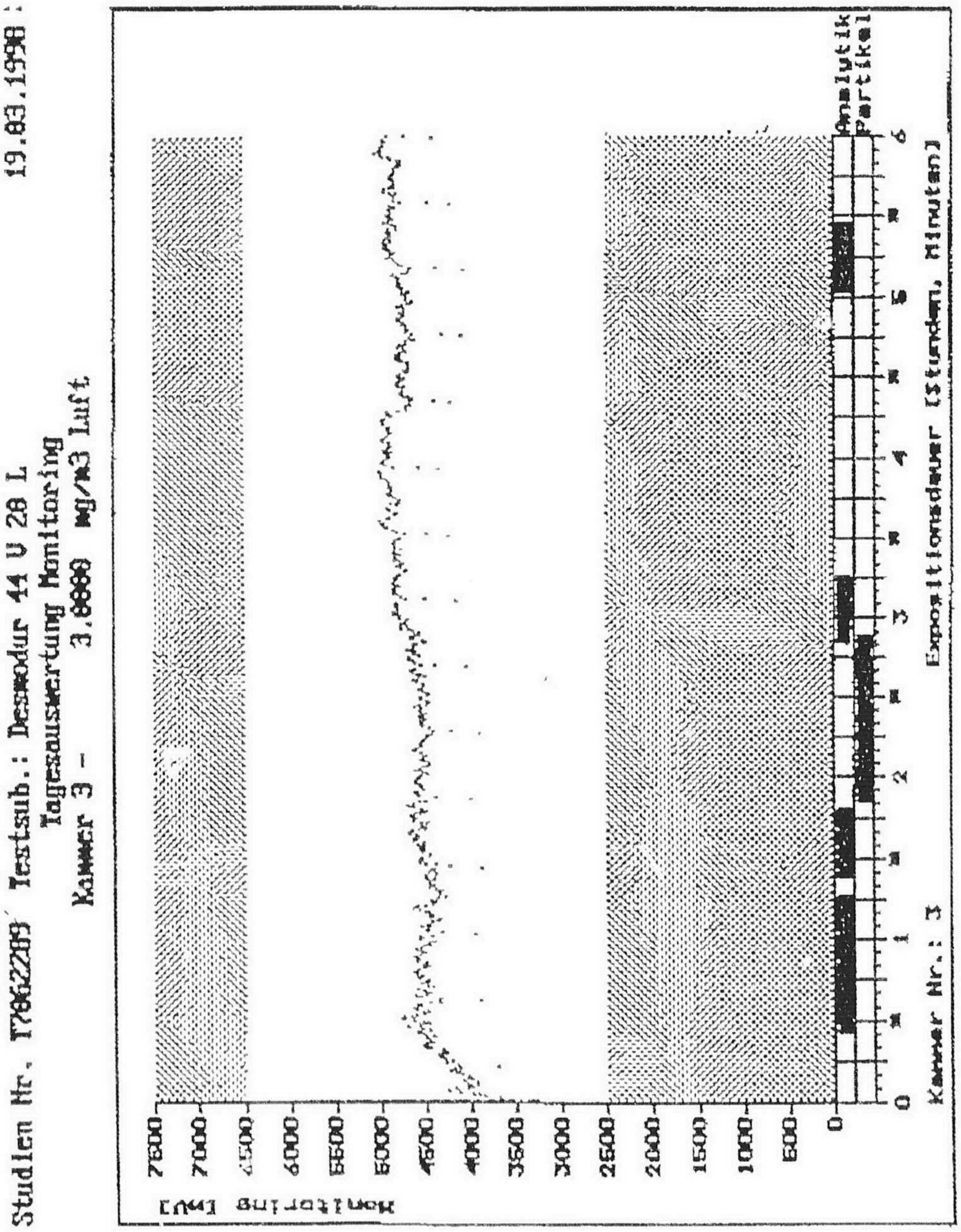
Monitoring of Atmosphere - 1 mg/m³



Legend (copy of raw data):

Ai: i-th analytical sample, Berner-Imp.: cascade impactor sampling
Beginn Exp.: start of exposure, Ende: End, time: mm.hh

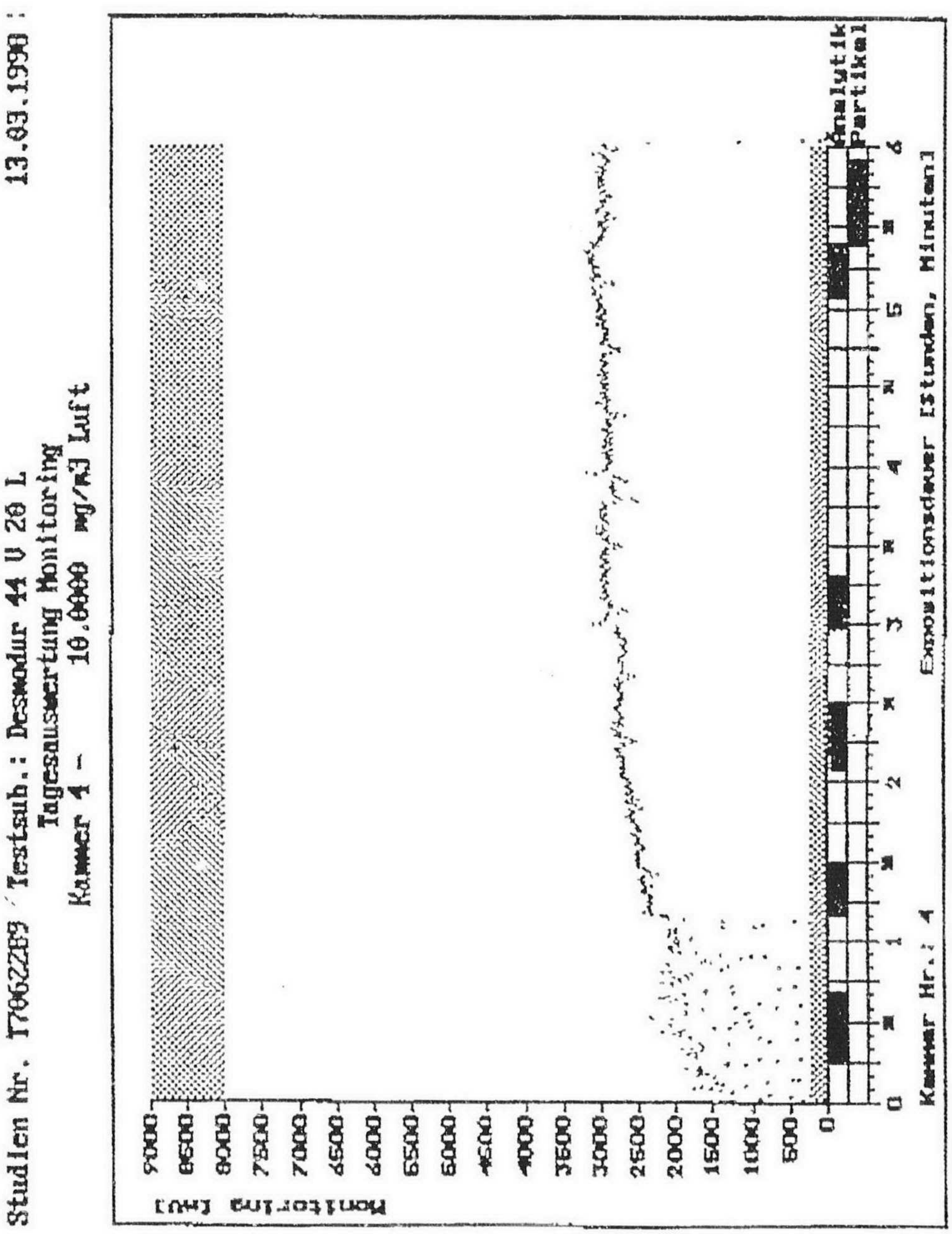
Monitoring of Atmosphere - 3 mg/m³



Legend (copy of raw data):

Ai: i-th analytical sample, Berner-Imp.: cascade impactor sampling
Beginn Exp.: start of exposure, Ende: End, time: mm.hh

Monitoring of Atmosphere - 10 mg/m³



Legend (copy of raw data):

Ai: i-th analytical sample, Berner-Imp.: cascade impactor sampling
Beginn Exp.: start of exposure, Ende: End, time: mm.hh

Body weights - Repeated Exposure

Analysis of Body Weights [all data in g]

Group 1: Control - FEMALES

	Postexposure Day 0	3	7	14	18
121	332.	339.	351.	379.	388.
122	331.	346.	354.	363.	378.
123	326.	338.	349.	352.	360.
124	318.	332.	349.	386.	406.
125	338.	351.	364.	390.	408.
126	355.	366.	384.	415.	442.
127	331.	341.	364.	382.	398.
128	325.	333.	350.	371.	355.
129	342.	352.	374.	412.	412.
130	333.	328.	334.	373.	374.
MEAN	333.1	342.6	357.3	382.3	392.1
STD	10.2	11.4	14.4	19.8	26.5

Group 2: 1 mg/m³ air - FEMALES

	Postexposure Day 0	3	7	14	18
131	331.	347.	357.	379.	413.
132	323.	343.	352.	369.	382.
133	349.	367.	400.	418.	429.
134	352.	334.	354.	390.	405.
135	318.	305.	325.	370.	397.
136	334.	326.	354.	375.	397.
137	307.	336.	351.	380.	365.
138	331.	359.	372.	394.	396.
139	342.	359.	378.	406.	394.
140	317.	333.	361.	383.	362.
MEAN	330.4	340.9	360.4	386.4	394.0
STD	14.5	18.3	19.8	15.9	20.4

Group 3: 3 mg/m³ air - FEMALES

	Postexposure Day				
	0	3	7	14	18
141	350.	369.	385.	392.	411.
142	331.	354.	356.	359.	397.
143	329.	337.	346.	358.	372.
144	315.	319.	334.	362.	374.
145	335.	342.	349.	379.	392.
146	343.	361.	380.	401.	410.
147	323.	319.	331.	378.	375.
148	352.	374.	377.	414.	426.
149	338.	352.	368.	410.	416.
150	327.	338.	346.	384.	387.
MEAN	334.3	346.5	357.2	383.7	396.0
STD	11.7	19.0	19.3	20.5	19.2

Group 4: 10 mg/m³ air - FEMALES

	Postexposure Day				
	0	3	7	14	18
151	340.	345.	377.	384.	404.
152	332.	338.	365.	372.	391.
153	294.	316.	335.	333.	349.
154	341.	343.	373.	386.	402.
155	315.	315.	331.	346.	349.
156	340.	348.	374.	383.	396.
157	333.	341.	360.	390.	403.
158	346.	352.	365.	386.	393.
159	316.	326.	342.	358.	373.
160	334.	348.	352.	382.	379.
MEAN	329.1	337.2	357.4	372.0	383.9
STD	16.0	13.5	16.6	19.6	21.0

Analysis of Body Weight Gains [all data in g]

Group 1: Control - FEMALES

	Postexposure Day 3	7	14	18
121	7.00	12.00	28.00	9.00
122	15.00	8.00	9.00	15.00
123	12.00	11.00	3.00	8.00
124	14.00	17.00	37.00	20.00
125	13.00	13.00	26.00	18.00
126	11.00	18.00	31.00	27.00
127	10.00	23.00	18.00	16.00
128	8.00	17.00	21.00	-16.00
129	10.00	22.00	38.00	.00
130	-5.00	6.00	39.00	1.00
MEAN	9.5	14.7	25.0	9.8
STD	5.7	5.7	12.3	12.4

Group 2: 1 mg/m³ air - FEMALES

	Postexposure Day 3	7	14	18
131	16.00	10.00	22.00	34.00
132	20.00	9.00	17.00	13.00
133	18.00	33.00	18.00	11.00
134	-18.00	20.00	36.00	15.00
135	-13.00	20.00	45.00	27.00
136	-8.00	28.00	21.00	22.00
137	29.00	15.00	29.00	-15.00
138	28.00	13.00	22.00	2.00
139	17.00	19.00	28.00	-12.00
140	16.00	28.00	22.00	-21.00
MEAN	10.5	19.5	26.0	7.6
STD	17.0	8.1	8.8	18.6

Group 3: 3 mg/m³ air - FEMALES

	Postexposure Day 3	7	14	18
141	19.00	16.00	7.00	19.00
142	23.00	2.00	3.00	38.00
143	8.00	9.00	12.00	14.00
144	4.00	15.00	28.00	12.00
145	7.00	7.00	30.00	13.00
146	18.00	19.00	21.00	9.00
147	-4.00	12.00	47.00	-3.00
148	22.00	3.00	37.00	12.00
149	14.00	16.00	42.00	6.00
150	11.00	8.00	38.00	3.00
MEAN	12.2	10.7	26.5	12.3
STD	8.6	5.8	15.2	11.0

Group 4: 10 mg/m³ air - FEMALES

	Postexposure Day 3	7	14	18
151	5.00	32.00	7.00	20.00
152	6.00	27.00	7.00	19.00
153	22.00	19.00	-2.00	16.00
154	2.00	30.00	13.00	16.00
155	.00	16.00	15.00	3.00
156	8.00	26.00	9.00	13.00
157	8.00	19.00	30.00	13.00
158	6.00	13.00	21.00	7.00
159	10.00	16.00	16.00	15.00
160	14.00	4.00	30.00	-3.00
MEAN	8.1	20.2	14.6	11.9
STD	6.3	8.6	10.2	7.4

ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of Day: 3 / FEMALES

```
Group-no.:  1
     7.000      15.000      12.000      14.000      13.000
    11.000      10.000       8.000      10.000      -5.000
   MEDIAN=   10.500   MEAN=      9.500   STD =     5.681

Group-no.:  2
    16.000      20.000      18.000     -18.000     -13.000
    -8.000      29.000      28.000      17.000      16.000
   MEDIAN=   16.500   MEAN=     10.500   STD =    17.011

Group-no.:  3
    19.000      23.000       8.000       4.000       7.000
    18.000      -4.000      22.000      14.000      11.000
   MEDIAN=   12.500   MEAN=     12.200   STD =     8.638

Group-no.:  4
     5.000       6.000      22.000       2.000        .000
     8.000       8.000       6.000      10.000      14.000
   MEDIAN=    7.000   MEAN=      8.100   STD =     6.262
```

BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

CALCULATED F	D.F.s	PROBABILITY
4.7170	3 & 2333.	.0032

HETEROGENEOUS VARIANCES (ONE-TAILED TEST)

ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB
TREATMENT	89.28	3	29.758	.273	.845
ERROR	3920.	36	108.88		
TOTAL	4009.	39			

NO OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL
NO STATISTICAL DIFFERENCE BETWEEN THE GROUPS

ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of Day: 7 / FEMALES

Group-no.: 1

12.000	8.000	11.000	17.000	13.000
18.000	23.000	17.000	22.000	6.000

MEDIAN= 15.000 MEAN= 14.700 STD = 5.658

Group-no.: 2

10.000	9.000	33.000	20.000	20.000
28.000	15.000	13.000	19.000	28.000

MEDIAN= 19.500 MEAN= 19.500 STD = 8.100

Group-no.: 3

16.000	2.000	9.000	15.000	7.000
19.000	12.000	3.000	16.000	8.000

MEDIAN= 10.500 MEAN= 10.700 STD = 5.813

Group-no.: 4

32.000	27.000	19.000	30.000	16.000
26.000	19.000	13.000	16.000	4.000

MEDIAN= 19.000 MEAN= 20.200 STD = 8.613

BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

CALCULATED F	D.F.s	PROBABILITY
.8081	3 & 2333.	.5079

HOMOGENEOUS VARIANCES (ONE-TAILED TEST)

ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB
TREATMENT	593.7	3	197.89	3.850	.017
ERROR	1850.	36	51.397		
TOTAL	2444.	39			

OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL

GAMES AND HOWELL MODIFICATION OF
TUKEY-KRAMERs HONESTLY SIGNIFICANT DIFFERENCE TEST
(WITH THE STUDENTIZED RANGE STATISTIC)

GROUPS COMPARED	CALCULATED TEST VALUE	DEGREES OF FREEDOM	PROBABILITY	CONCLUSION
5. % ONE-TAILED TEST				
1 AND 2	2.17	16	.4403	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 2	2.17	16	.4403	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
1 AND 3	-2.21	18	.4251	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 3	2.21	18	.4251	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
1 AND 4	2.39	16	.3616	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 4	2.39	16	.3616	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
2 AND 3	-3.95	16	.0571	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
2 AND 3	3.95	16	.0571	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
2 AND 4	.26	18	.9976	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
2 AND 4	.26	18	.9976	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
3 AND 4	4.09	16	.0472	SIGNIFICANT
5. % TWO-TAILED TEST				
3 AND 4	4.09	16	.0472	NOT SIGNIFICANT

ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of Day: 14 / FEMALES

```
Group-no.:  1
     28.000        9.000       3.000      37.000       26.000
     31.000       18.000      21.000      38.000       39.000
  MEDIAN=    27.000   MEAN=     25.000    STD =     12.293

Group-no.:  2
     22.000       17.000      18.000      36.000       45.000
     21.000       29.000      22.000      28.000       22.000
  MEDIAN=    22.000   MEAN=     26.000    STD =      8.769

Group-no.:  3
      7.000        3.000      12.000      28.000       30.000
     21.000       47.000      37.000      42.000       38.000
  MEDIAN=    29.000   MEAN=     26.500    STD =     15.241

Group-no.:  4
      7.000        7.000      -2.000      13.000       15.000
      9.000       30.000      21.000      16.000       30.000
  MEDIAN=    14.000   MEAN=     14.600    STD =     10.233
```

BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

CALCULATED F	D.F.s	PROBABILITY
.9821	3 & 2333.	.5985

HOMOGENEOUS VARIANCES (ONE-TAILED TEST)

ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB
TREATMENT	958.1	3	319.36	2.261	.097
ERROR	5085.	36	141.25		
TOTAL	6043.	39			

NO OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL
NO STATISTICAL DIFFERENCE BETWEEN THE GROUPS

ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of Day: 18 / FEMALES

Group-no.: 1

9.000	15.000	8.000	20.000	18.000
27.000	16.000	-16.000	.000	1.000

MEDIAN= 12.000 MEAN= 9.800 STD = 12.363

Group-no.: 2

34.000	13.000	11.000	15.000	27.000
22.000	-15.000	2.000	-12.000	-21.000

MEDIAN= 12.000 MEAN= 7.600 STD = 18.620

Group-no.: 3

19.000	38.000	14.000	12.000	13.000
9.000	-3.000	12.000	6.000	3.000

MEDIAN= 12.000 MEAN= 12.300 STD = 10.955

Group-no.: 4

20.000	19.000	16.000	16.000	3.000
13.000	13.000	7.000	15.000	-3.000

MEDIAN= 14.000 MEAN= 11.900 STD = 7.355

BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

CALCULATED F	D.F.s	PROBABILITY
2.4401	3 & 2333.	.0614

HOMOGENEOUS VARIANCES (ONE-TAILED TEST)

ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB
TREATMENT	140.6	3	46.867	.278	.842
ERROR	6063.	36	168.42		
TOTAL	6204.	39			

NO OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL
NO STATISTICAL DIFFERENCE BETWEEN THE GROUPS

Lung Weight - Repeated Exposure

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          ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA
-----------------------------------------------------------------
Analysis of LUNG WEIGHT - absolute/FEMALES
-----------------------------------------------------------------

 Group-no.:  1 (121 - 130)
     2486.000    2593.000    2384.000    2681.000    2517.000
     2748.000    2566.000    2556.000    2552.000    2226.000
     MEDIAN=  2554.000   MEAN=  2530.900   STD =   146.279

 Group-no.:  2 (131 - 140)
     2892.000    2541.000    2650.000    2420.000    2502.000
     2766.000    2261.000    2962.000    2769.000    2814.000
     MEDIAN=  2708.000   MEAN=  2657.700   STD =   223.068

 Group-no.:  3 (141-150)
     2684.000    2469.000    2510.000    2670.000    2812.000
     2769.000    2589.000    2860.000    2578.000    2482.000
     MEDIAN=  2629.500   MEAN=  2642.300   STD =   139.427

 Group-no.:  4 (15[illegible]-160)
     3200.000    [illegible]00.000    2998.000    2911.000    2918.000
     3191.000    2843.000    2479.000    3092.000    2945.000
     MEDIAN=  2971.500   MEAN=  2977.700   STD =   219.529
-----------------------------------------------------------------
   BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

  CALCULATED F        D.F.s             PROBABILITY
  ------------   ---------------       -----------
     1.0715         3 &   2333.            .3604

   HOMOGENEOUS VARIANCES (ONE-TAILED TEST)
-----------------------------------------------------------------

   ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE
-----------------------------------------------------------------
 SOURCE         SS           DF         MS            F          PROB
-----------------------------------------------------------------
 TREATMENT   1.1081E+06      3     3.69374E+05  10.646         .000
 ERROR       1.2491E+06     36       34697.
 TOTAL       2.3572E+06     39
-----------------------------------------------------------------
   OVERALL SIGNIFICANCE AT  5.% (ONE-TAILED) LEVEL
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GAMES AND HOWELL MODIFICATION OF
TUKEY-KRAMERs HONESTLY SIGNIFICANT DIFFERENCE TEST
(WITH THE STUDENTIZED RANGE STATISTIC)

GROUPS COMPARED	CALCULATED TEST VALUE	DEGREES OF FREEDOM	PROBABILITY	CONCLUSION
5. % ONE-TAILED TEST				
1 AND 2	2.13	16	.4585	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 2	2.13	16	.4585	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
1 AND 3	2.47	18	.3316	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 3	2.47	18	.3316	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
1 AND 4	7.57	16	.0003	SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 4	7.57	16	.0003	SIGNIFICANT
5. % ONE-TAILED TEST				
2 AND 3	-.26	15	.9976	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
2 AND 3	.26	15	.9976	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
2 AND 4	4.57	18	.0218	SIGNIFICANT
5. % TWO-TAILED TEST				
2 AND 4	4.57	18	.0218	SIGNIFICANT
5. % ONE-TAILED TEST				
3 AND 4	5.77	15	.0049	SIGNIFICANT
5. % TWO-TAILED TEST				
3 AND 4	5.77	15	.0049	SIGNIFICANT

ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of LUNG WEIGHT - relative to BODY WEIGHT/FEMALES

Group-no.: 1 (121-130)

640.722	685.979	662.222	660.345	616.912
621.719	644.724	720.000	619.417	595.187

MEDIAN= 642.723 MEAN= 646.723 STD = 36.956

Group-no.: 2 (131-140)

700.242	665.183	617.716	597.531	630.227
696.725	619.452	747.980	702.792	777.348

MEDIAN= 680.954 MEAN= 675.520 STD = 59.768

Group-no.: 3 (141-150)

653.041	621.914	674.731	713.904	717.347
675.366	690.400	671.362	619.712	641.344

MEDIAN= 673.047 MEAN= 667.912 STD = 34.229

Group-no.: 4 (151-160)

792.079	818.414	859.026	724.129	836.103
805.808	705.459	630.789	828.954	777.045

MEDIAN= 798.943 MEAN= 777.781 STD = 70.693

BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

CALCULATED F	D.F.s	PROBABILITY
2.1046	3 & 2333.	.0961

HOMOGENEOUS VARIANCES (ONE-TAILED TEST)

ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB
TREATMENT	1.0260E+05	3	34201.	12.317	.000
ERROR	9.9964E+04	36	2776.8		
TOTAL	2.0257E+05	39			

OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL

GAMES AND HOWELL MODIFICATION OF
TUKEY-KRAMERs HONESTLY SIGNIFICANT DIFFERENCE TEST
(WITH THE STUDENTIZED RANGE STATISTIC)

GROUPS COMPARED	CALCULATED TEST VALUE	DEGREES OF FREEDOM	PROBABILITY	CONCLUSION
5. % ONE-TAILED TEST				
1 AND 2	1.83	15	.5792	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 2	1.83	15	.5792	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
1 AND 3	1.88	18	.5566	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 3	1.88	18	.5566	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
1 AND 4	7.35	14	.0007	SIGNIFICANT
5. % TWO-TAILED TEST				
1 AND 4	7.35	14	.0007	SIGNIFICANT
5. % ONE-TAILED TEST				
2 AND 3	-.49	14	.9848	NOT SIGNIFICANT
5. % TWO-TAILED TEST				
2 AND 3	.49	14	.9848	NOT SIGNIFICANT
5. % ONE-TAILED TEST				
2 AND 4	4.94	18	.0126	SIGNIFICANT
5. % TWO-TAILED TEST				
2 AND 4	4.94	18	.0126	SIGNIFICANT
5. % ONE-TAILED TEST				
3 AND 4	6.26	13	.0034	SIGNIFICANT
5. % TWO-TAILED TEST				
3 AND 4	6.26	13	.0034	SIGNIFICANT

```
          ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA
-----------------------------------------------------------------
Analysis of BODY WEIGHT/FEMALES
-----------------------------------------------------------------

 Group-no.:  1 (121-130)
      388.000      378.000      360.000      406.000      408.000
      442.000      398.000      355.000      412.000      374.000
     MEDIAN=  393.000  MEAN=  392.100   STD =    26.493

 Group-no.:  2 (131-140)
      413.000      382.000      429.000      405.000      397.000
      397.000      365.000      396.000      394.000      362.000
     MEDIAN=  396.500  MEAN=  394.000   STD =    20.380

 Group-no.:  3 (141-150)
      411.000      397.000      372.000      374.000      392.000
      410.000      375.000      426.000      416.000      387.000
     MEDIAN=  394.500  MEAN=  396.000   STD =    19.206

 Group-no.:  4 (151-160)
      404.000      391.000      349.000      402.000      349.000
      396.000      403.000      393.000      373.000      379.000
     MEDIAN=  392.000  MEAN=  383.900   STD =    20.963
-----------------------------------------------------------------
   BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.0500) LEVEL

  CALCULATED F       D.F.s            PROBABILITY
  ------------  ---------------       ------------
     .3662         3 &   2333.           .7805

   HOMOGENEOUS VARIANCES (ONE-TAILED TEST)
-----------------------------------------------------------------

   ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE
-----------------------------------------------------------------
 SOURCE          SS          DF       MS            F         PROB
-----------------------------------------------------------------
 TREATMENT   846.2            3      282.07        .586       .632
 ERROR       1.7330E+04      36      481.38
 TOTAL       1.8176E+04      39
-----------------------------------------------------------------
   NO OVERALL SIGNIFICANCE AT  5.% (ONE-TAILED) LEVEL
   NO STATISTICAL DIFFERENCE BETWEEN THE GROUPS
```

Gross Necropsy

Individual findings / female guinea pigs (Repeated Exposure)

Group	Animal No.	Time of death	Sacrificed after	Pathology findings
13 (air control)	121		18 d	no observable findings
	122		18 d	no observable findings
	123		18 d	no observable findings
	124		18 d	no observable findings
	125		18 d	no observable findings
	126		18 d	no observable findings
	127		18 d	no observable findings
	128		18 d	no observable findings
	129		18 d	**lung**: dark red foci
	130		18 d	no observable findings
14 (1 mg/ m^3)	131		18 d	no observable findings
	132		18 d	no observable findings
	133		18 d	**lung**: dark red foci
	134		18 d	no observable findings
	135		18 d	no observable findings
	136		18 d	no observable findings
	137		18 d	no observable findings
	138		18 d	no observable findings
	139		18 d	no observable findings
	140		18 d	no observable findings
15 (3 mg/ m^3)	141		18 d	no observable findings
	142		18 d	no observable findings
	143		18 d	no observable findings
	144		18 d	no observable findings
	145		18 d	no observable findings
	146		18 d	no observable findings
	147		18 d	no observable findings
	148		18 d	**lung**: dark red foci
	149		18 d	no observable findings
	150		18 d	no observable findings

Group	Animal No.	Time of death	Sacrificed after	Pathology findings
16 (10 mg/ m^3)	151		18 d	**lung**: less collapsed
	152		18 d	**lung**: less collapsed
	153		18 d	**lung**: less collapsed
	154		18 d	**lung**: dark red areas; less collapsed; firm consistency **lung-associated lymph nodes**: enlarged **stomach**: bloated **heard**: congestion of vessels, left ventricle
	155		18 d	**lung-associated lymph nodes**: enlarged
	156		18 d	**lung**: less collapsed **lung-associated lymph nodes**: enlarged
	157		18 d	**lung**: dark red foci **lung-associated lymph nodes**: enlarged
	158		18 d	**lung-associated lymph nodes**: enlarged
	159		18 d	**lung**: less collapsed **lung-associated lymph nodes**: enlarged
	160		18 d	**lung**: less collapsed **lung-associated lymph nodes**: enlarged

Incidence Table - Macroscopic Lung Findings

Macroscopic lung findings (including lung-associated lymph nodes)

```
R X C CHI-SQUARE - TEST:
------------------------
 Chisquare =    27.692  DF =  3  Frequency =     3.250
 Chi-Tab. =      7.815  p = 0.05 (bilateral)

FISHERs EXACT TEST:
-----------------------------------------------------
 Group: 1                    Incidence:  1/10    B  U
 Group: 2     P=  .7632      Incidence:  1/10    -  -
 Group: 3     P=  .7632      Incidence:  1/10    -  -
 Group: 4     P=  .0001      Incidence: 10/10   ** ++
-----------------------------------------------------


B = bilateral comparison of groups
U = unilateral comparison of groups
P = single-tailed probability

#/# = 1st figure  number of positive observations
      2nd figure: number of total observations
```

Pulmonary Function Measurements

RD50 Evaluation

Print-Date: 27.05.1998

Statistics printout

Group designation: 0 mg/cbm

Measuring results	Means[abs]	SD[abs]	Min[%]	Max[%]
Peak Inspiratory Flow [ml/min]:	14.8	0.9	65.3	116.8
Peak Expiratory Flow [ml/min]:	11.4	0.9	56.6	113.4
Tidal Volume [ml]:	2.7	0.2	80.8	134.4
Minute Volume [ml/min]:	268.9	15.9	61.3	113.6
Respiratory Rate [breaths/min]:	107.1	8.9	77.0	117.4
Expiratory Time [msec]:	324.7	21.1	90.9	128.1
Inspiratory Time [msec]:	261.2	15.9	92.2	129.1
Apnea Time [msec]:	7.6	3.4	69.5	404.1
Apnea Logging Period [#]:	0.8	0.5	36.3	468.2
ET/IT:	1.2	0.0	93.1	110.3
PIF/PEF:	1.3	0.1	91.8	120.2
PEF*(IT+ET)/TV * 1/1000:	2.5	0.1	86.0	108.6
TV/IT:	0.0	0.0	65.4	113.0

abs: absolute data for adaptation period
%: rel. change to adaptation period

E 01

RD50 Evaluation

Print-Date: 27.05.1998

Statistics printout

Group designation: 3 mg/cbm

Measuring results	Means[abs]	SD[abs]	Min[%]	Max[%]
Peak Inspiratory Flow [ml/min]:	13.8	1.1	62.0	121.7
Peak Expiratory Flow [ml/min]:	11.0	0.9	46.6	117.6
Tidal Volume [ml]:	2.5	0.2	56.3	142.0
Minute Volume [ml/min]:	255.1	17.3	52.9	128.7
Respiratory Rate [breaths/min]:	103.3	9.1	81.7	113.1
Expiratory Time [msec]:	329.0	21.6	90.6	116.9
Inspiratory Time [msec]:	264.8	18.1	88.7	122.5
Apnea Time [msec]:	7.9	4.4	58.6	450.9
Apnea Logging Period [#]:	0.9	1.6	17.3	741.8
ET/IT:	1.2	0.0	91.7	108.7
PIF/PEF:	1.3	0.1	95.6	136.0
PEF*(IT+ET)/TV * 1/1000:	2.6	0.1	79.9	104.2
TV/IT:	0.0	0.0	59.8	128.3

abs: absolute data for adaptation period
%: rel. change to adaptation period

RD50 Evaluation

Print-Date: 27.05.1998

Statistics printout

Group designation: 10 mg/cbm

Measuring results	Means[abs]	SD[abs]	Min[%]	Max[%]
Peak Inspiratory Flow [ml/min]:	14.0	0.9	70.1	109.2
Peak Expiratory Flow [ml/min]:	11.1	1.0	59.1	113.5
Tidal Volume [ml]:	2.7	0.2	70.2	116.1
Minute Volume [ml/min]:	268.5	19.1	63.7	113.3
Respiratory Rate [breaths/min]:	100.3	5.4	82.7	114.7
Expiratory Time [msec]:	322.9	14.4	88.4	116.6
Inspiratory Time [msec]:	280.8	16.1	90.4	123.4
Apnea Time [msec]:	6.7	2.9	69.9	346.4
Apnea Logging Period [#]:	0.6	0.8	29.8	511.1
ET/IT:	1.2	0.1	91.5	112.2
PIF/PEF:	1.3	0.1	93.1	123.1
PEF*(IT+ET)/TV * 1/1000:	2.5	0.1	87.5	102.9
TV/IT:	0.0	0.0	69.2	113.8

abs: absolute data for adaptation period
%: rel. change to adaptation period

E 03

RD50 Evaluation

Print-Date: 27.05.1998

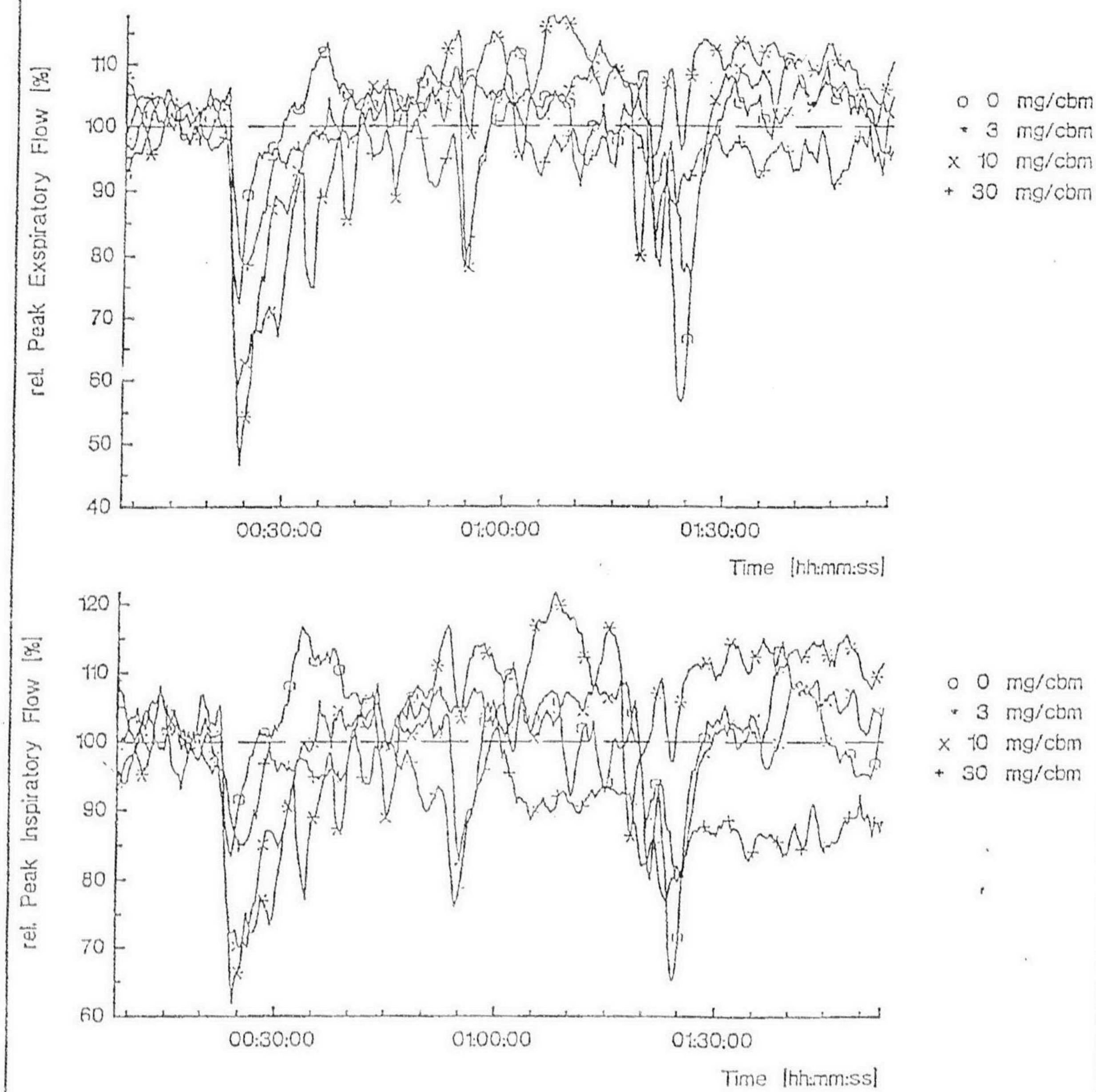
Statistics printout

Group designation: 30 mg/cbm

Measuring results	Means[abs]	SD[abs]	Min[%]	Max[%]
Peak Inspiratory Flow [ml/min]:	15.6	0.9	76.2	108.2
Peak Expiratory Flow [ml/min]:	12.0	0.6	78.1	106.1
Tidal Volume [ml]:	2.8	0.1	79.4	104.2
Minute Volume [ml/min]:	291.2	14.3	84.5	109.0
Respiratory Rate [breaths/min]:	106.9	5.8	85.5	128.2
Expiratory Time [msec]:	316.6	14.5	75.0	115.8
Inspiratory Time [msec]:	255.0	10.7	84.1	112.8
Apnea Time [msec]:	6.0	1.7	75.2	158.7
Apnea Logging Period [#]:	0.4	0.3	45.4	386.2
ET/IT:	1.2	0.0	84.1	107.4
PIF/PEF:	1.3	0.0	86.8	110.8
PEF*(IT+ET)/TV * 1/1000:	2.4	0.1	92.1	104.4
TV/IT:	0.0	0.0	81.3	105.8

abs: absolute data for adaptation period
%: rel. change to adaptation period

E 04



Print-Date: 27.05.1998

RD50

T7062289

Desmodur 44 V 20 L

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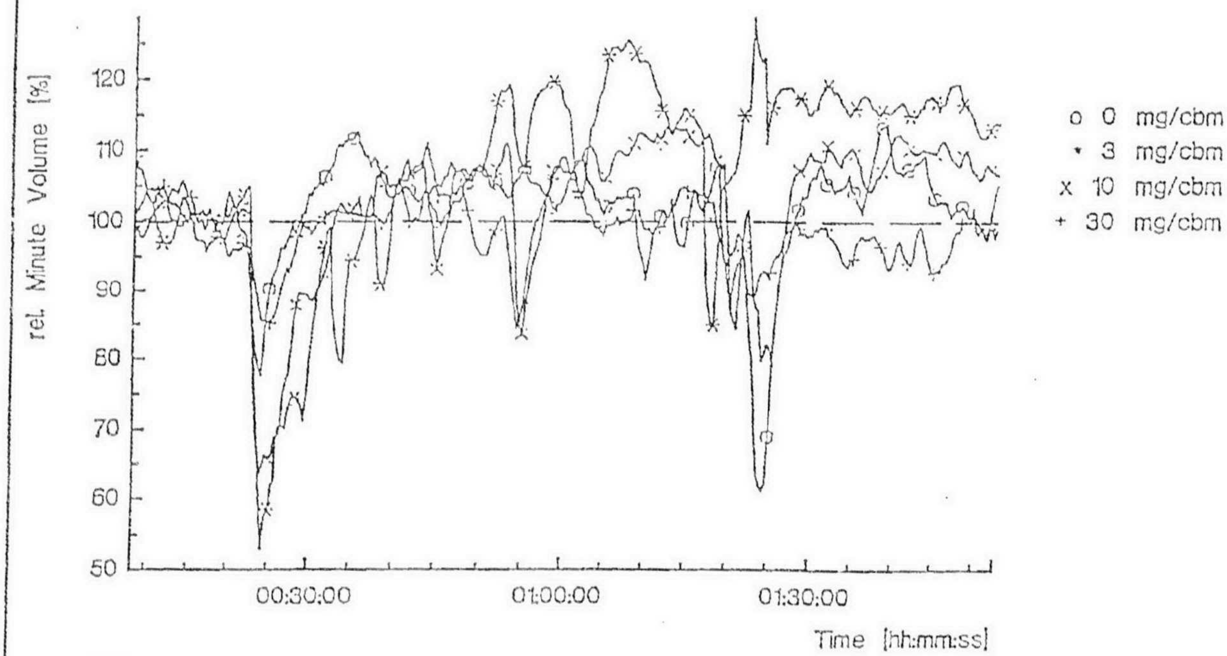

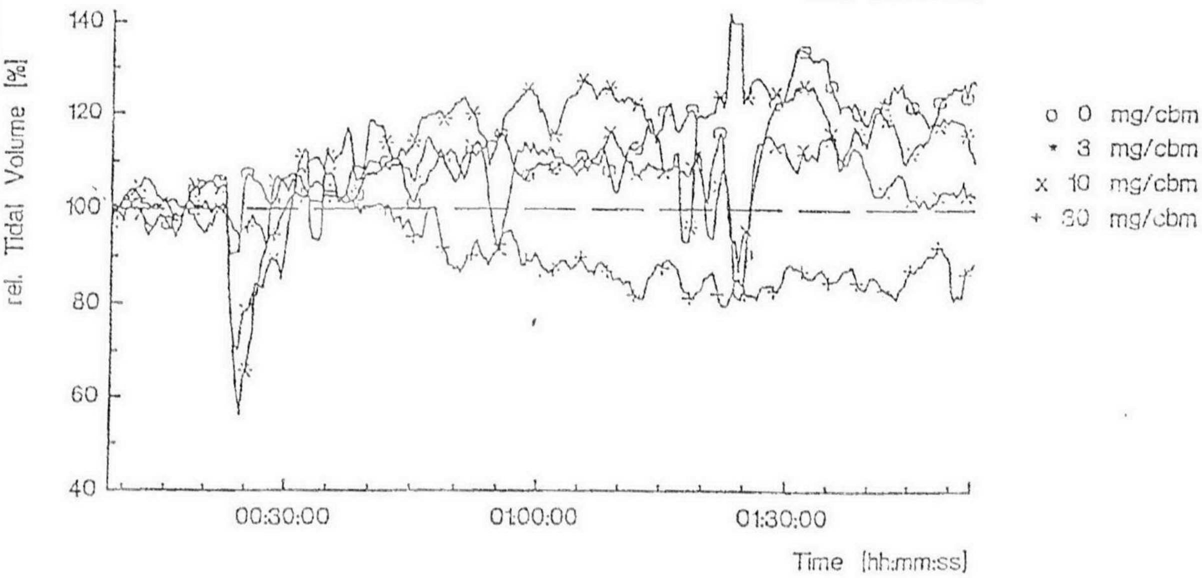

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Desmodur 44 V 20 L

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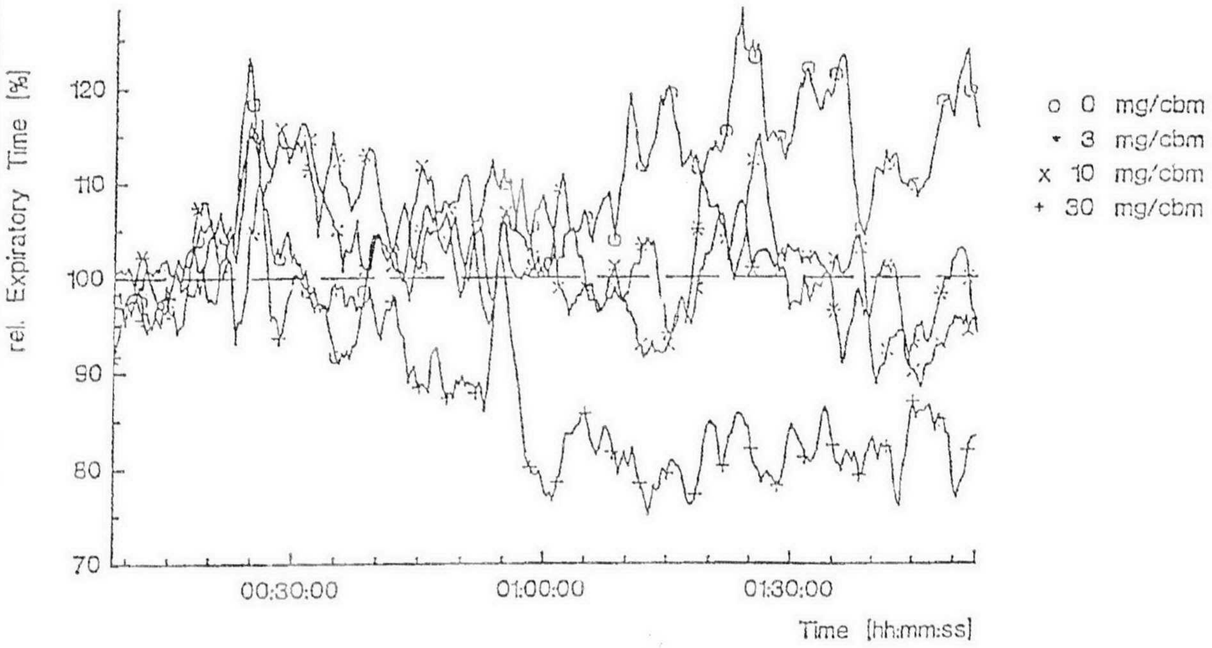

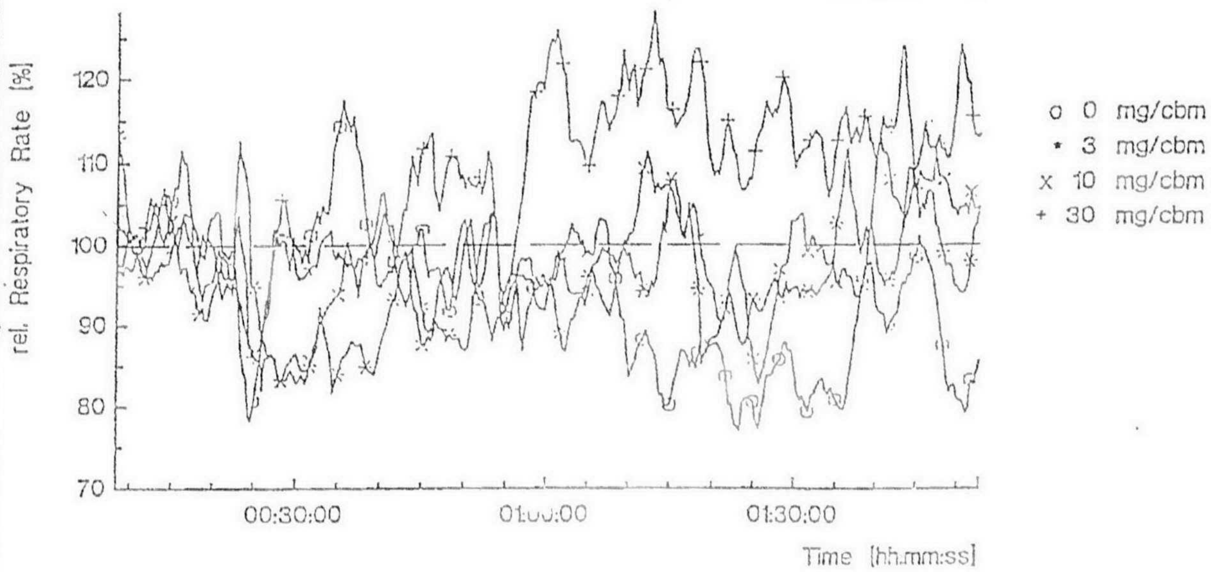

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Desmodur 44 V 20 L

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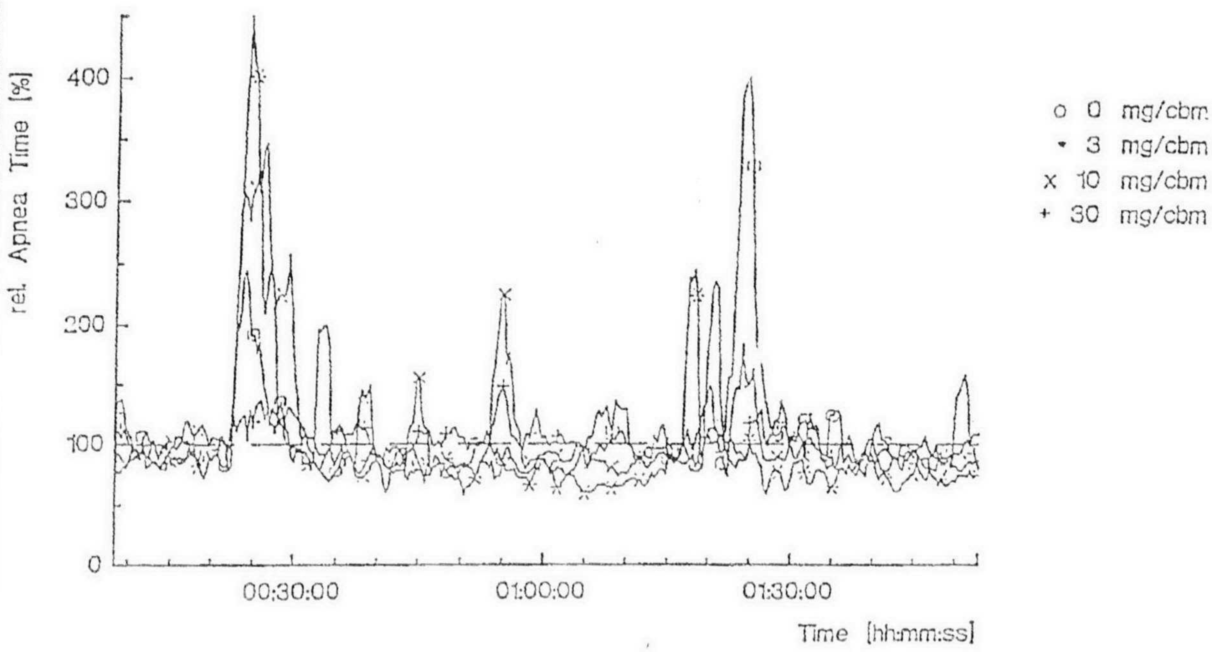

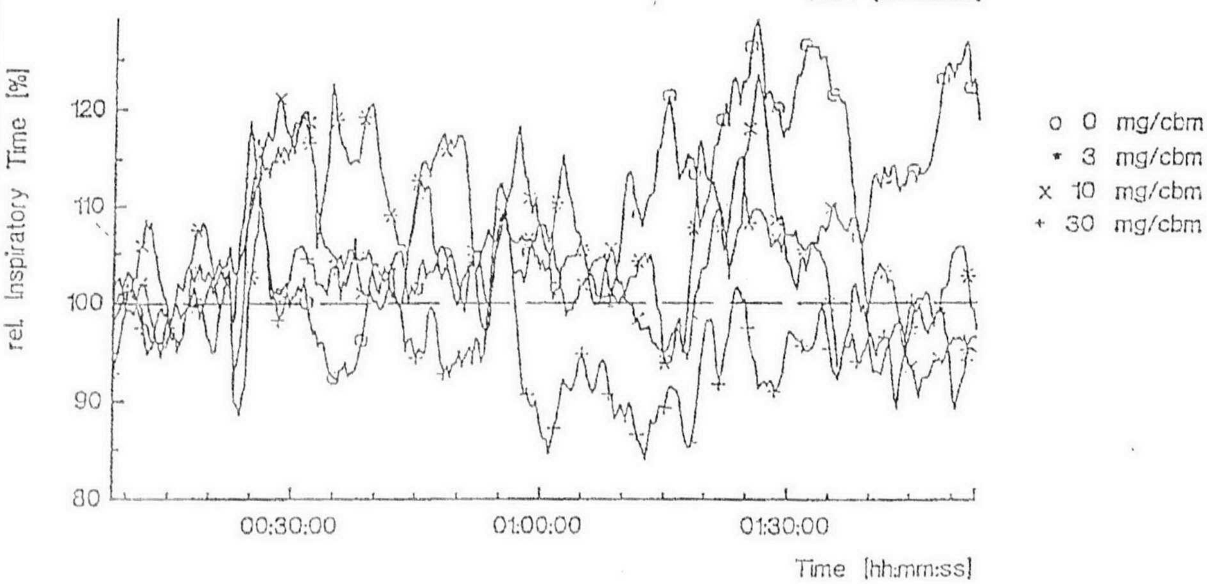

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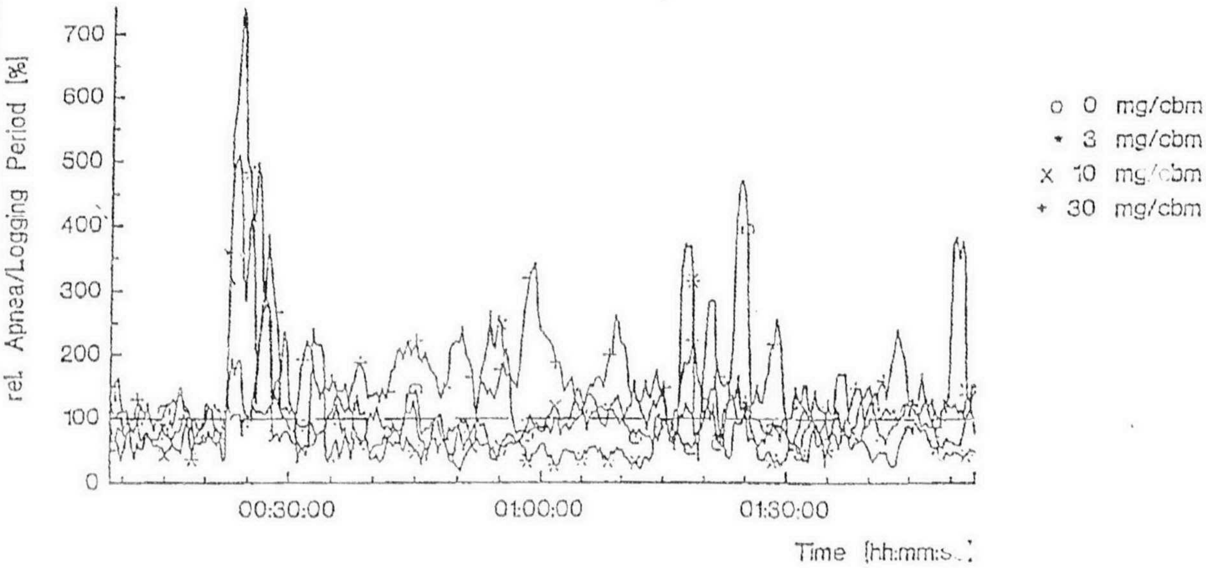

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Desmodur 44 V 20 L

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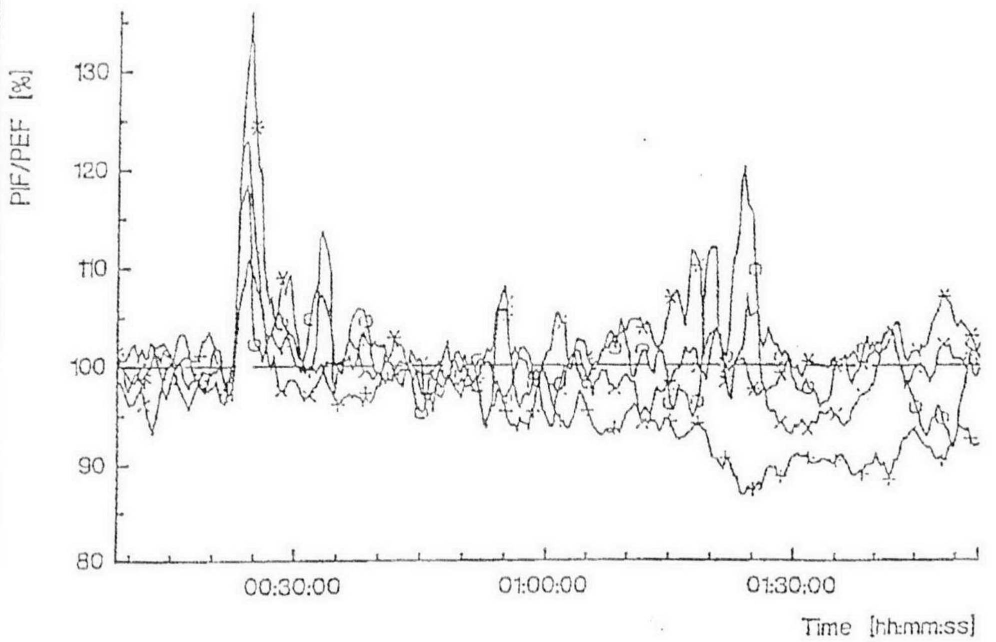

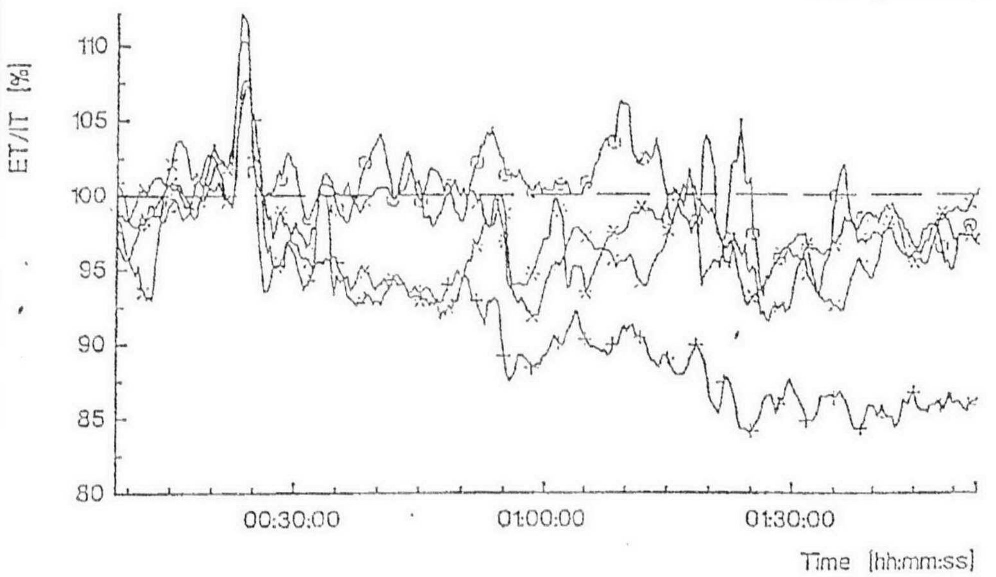

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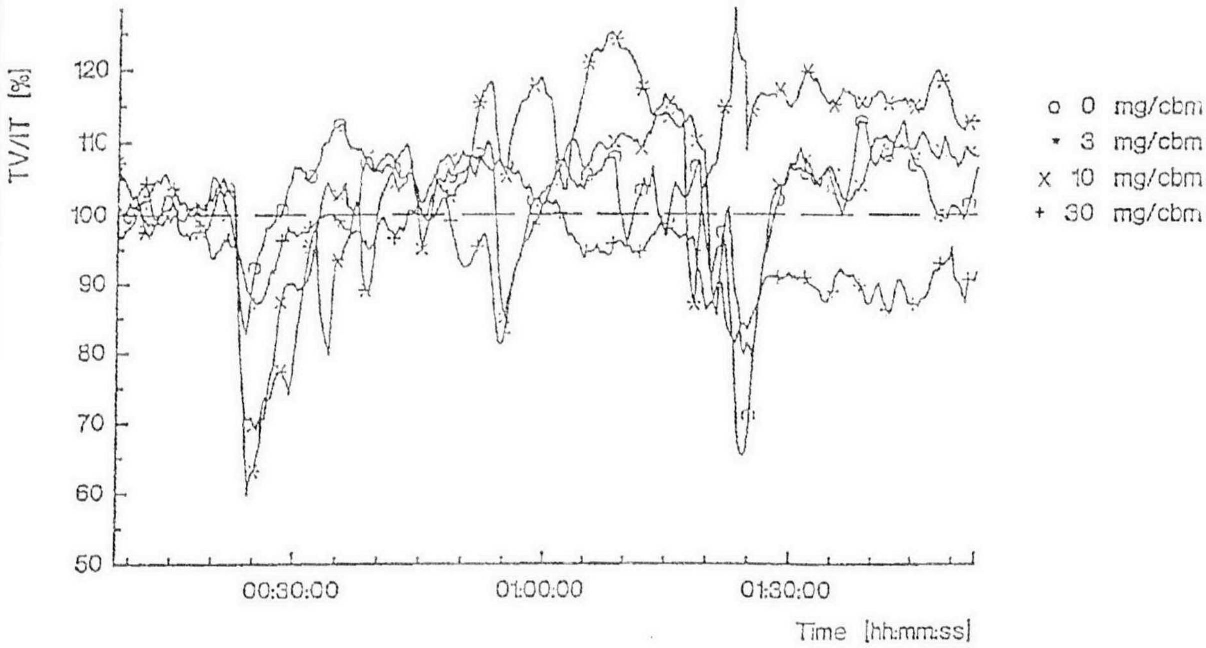
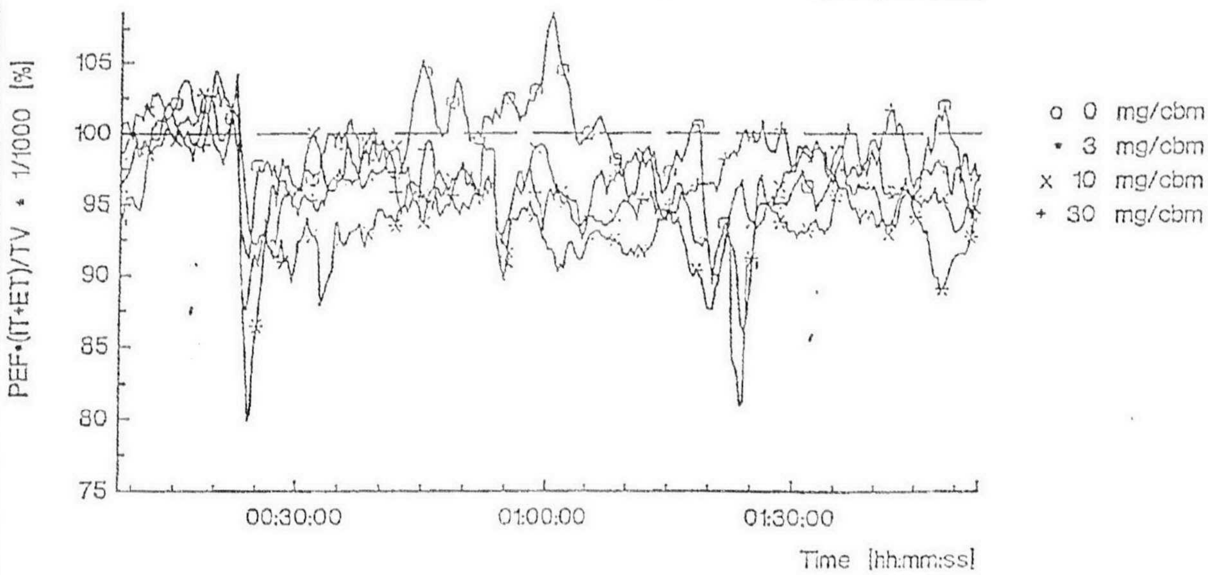
Desmodur 44 V 20 L





Print-Date: 27.05.1996

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T7062289

Desmodur 44 V 20 L

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Chow Specification - Nutrients

Altromin Standard Diets 3020 / Totally Pathogene Free TPF®

ALTROMIN 3020 Guinea Pig Maintenance Diet is an established maintenance diet for animals of 10 weeks and older. The diet should be offered ad libitum together with an ample supply of fresh water.
Sealed in polyethylene lined sacks, ALTROMIN 3020 can be passed directly into the SPF facility following surface disinfection.

Room Temperature: 20 - 24 °C (EG recommended); Relative Humidity: 50-60%

Food Absorption g/day

	growth phase	adult
Guinea Pigs	up to 50 g	ca. 60 g

ALTROMIN 3020 is available in:

	Powder	3021
3.0 mm	Pellets	3022
15.0 mm	Pellets	3025

Specification of Maintenance Diet Guinea Pigs:

Nutrients (average % content in the diet)	
Crude protein	17.0
Crude fat	3.75
Crude fiber	13.5
Ash	8.2
Moisture	12.0
Nitrogen-free extract	42.7

Metabolizable Energy (calculated)	
Kcal/kg	2450.0
MJ/kg	10.2

Minerals (average % content in the diet)	
Calcium	0.95
Phosphorus	0.7
Magnesium	0.2
Sodium	0.2
Potassium	1.5

Amino Acids (average % content in the diet)	
Lysine	0.90
Methionine	0.25
Cystine	0.25
Phenylalanine	0.70
Tyrosine	0.60
Arginine	1.00
Histidine	0.40
Tryptophane	0.20
Threonine	0.70
Isoleucine	0.90
Leucine	1.40
Valine	0.90

Trace elements (average mg content in 1 kg diet)	
Manganese	62.0
Iron	165.0
Copper	5.0
Zinc	50.0
Iodine	0.9
Fluorine	10.0

Vitamins (additive in 1 kg diet)	Standard-Diet	Standard-Diet fortified
Vitamin A	15000.0 IU	25000.0 IU
Vitamin D_3	600.0 IU	1000.0 IU
Vitamin E	75.0 mg	125.0 mg
Vitamin K_3	3.0 mg	5.0 mg
Vitamin B_1	18.0 mg	30.0 mg
Vitamin B_2	12.0 mg	20.0 mg
Vitamin B_6	9.0 mg	15.0 mg
Vitamin B_{12}	24.0 mcg	40.0 mcg
Nicotinic acid	36.0 mg	60.0 mg
Pantothenic acid	21.0 mg	35.0 mg

Folic acid	2.0 mg	3.0 mg
Biotin	60.0 mcg	100.0 mcg
Choline	600.0 mg	1000.0 mg
Vitamin C	1036.0 mg	1060.0 mg

Chow Specification - Impurities

Impurity	Max. acceptable value	LUFA - Limit of detection	Altromin *
Aflatoxine B1 / B2	0.01	0.0025	nng
Aflatoxine G1 / G2	0.01	0.0025	nng
Antibiotic activity	± 0		nng
Arsenic	2.0	0.2	0.3
Fluoride	150.0	5.0	22.0
Mercury	0.1	0.01	0.08
Lead	5.0	0.1	0.37
Cadmium		0.01	0.10
Selenium		0.10	1.0
[illegible]ene		0.001	< 0.001
Quintozene		0.001	< 0.001
HCB (Hexachlorbenzene)		0.001	< 0.001
α -HCH		0.001	< 0.001
β -HCH		0.002	< 0.002
τ -HCH	0.1	0.001	0.002
Heptachlor	0.03	0.005	< 0.005
Heptachlorepoxid	0.03	0.005	< 0.005
α - Chlordan	0.05	0.005	< 0.005
τ - Chlordan	0.05	0.005	< 0.005
Aldrin	0.02	0.005	< 0.005
Dieldrin	0.02	0.005	< 0.005
Endrin	0.02	0.01	< 0.01
o,p - DDE	0.05	0.005	< 0.005
p,p - DDE	0.05	0.005	< 0.005
o,p - DDD	0.05	0.005	< 0.005
o,p - DDT	0.05	0.005	< 0.005
p,p - DDD	0.05	0.01	< 0.01
p,p - DDT	0.05	0.01	< 0.01
Methoxychlor		0.01	< 0.01
PCB qual,			nng
Chlorthion		0.01	< 0.01
Disulfothion		0.005	< 0.005
Malathion		0.01	< 0.01
Methylparathion		0.005	< 0.005
Ethylparathion		0.01	< 0.01
Sulfotepp		0.002	< 0.002
Fenthion		0.005	< 0.005
Diazinon		0.01	< 0.01
Dibrom		0.02	< 0.02
Dimethoate		0.005	< 0.005
Trichlorphon		0.01	< 0.01
Fenitrothion		0.01	< 0.01

* In this study Altromin 3022 was used. 2 is the degree of pelletation, dimension: ppm

Tap Water Specification

No.	Substance	Limit mg/l	computed as	equivalent mmol/m³	acceptable error of value (± mg/l)
1	Arsenic	0.04	As	0.5	0.015
2	Lead	0.04	Pb	0.2	0.02
3	Cadmium	0.005	Cd	0.04	0.002
4	Chrome	0.05	Cr	1	0.01
5	Cyanide	0.05	CN-	2	0.01
6	Fluoride	1.5	F-	79	0.2
7	Nickel	0.05	Ni	0.9	0.01
8	Nitrate	50	NO_3^-	806	2
9	Nitrite	0.1	NO_2^-	2.2	0.02
10	Mercury	0.001	Hg	0.005	0.0005
11	Polycyclic aromatic carbohyrates - Fluoranthene - Benzo-b-fluoranthene - Benzo-k-fluoranthene - Benzo-a-pyrene - Benzo-(ghi)-perylene - Indeno-(1,2,3-cd)-pyrene	sum 0.0002	C	0.02	0.00004
12	Organochloric compounds - 1,1,1-Trichlorethane - Trichlorethylene - Tetrachlorethylene - Dichlormethane	sum 0.01	-	-	0.004
	- Tetrachlormethane	0.003	CCl4	0.02	0.001
13	a. Pesticides b. Polychlorinated Polybromated biphenyles and terphenyles	indiv-dual com-pound 0.0001	-		0.00005
		sum 0.0005	-	-	0.0002
14	Antimony	0.01	Sb	0.08	0.002
15	Selenium	0.01	Se	0.13	0.002

F 01

Test Substance - Certificate

WERKSPRÜFZEUGNIS (EN 10204-2.3)

Bayer

Bayer AG
PU-Produktion Uerdingen

47812 Krefeld

Hr. Pilger
PU-Stab
B 211

DATUM 22.10.97

Leverkusen

ARTIKEL ARTIKELNAME
02 00235672 DESMODUR 44 V 20 L

IHRE BESTELLNUMMER/PRODUKTNUMMER
211079

PARTIE	MENGE	AUFTRAG-AAW
7920/L2D	1 KG	0895272H7

ES WURDE VORSCHRIFTSMAESSIG PROBE GENOMMEN UND MIT FOLGENDEM ERGEBNIS GEPRUEFT:

PRUEFUNGEN	ERGEBNISSE	SPEZIFIKATION
1. 2011-0248603-94 NCO-Gehalt	31.68	30.50 - 32.50 %
2. 2011-0313703-95 Viskosität (25°C)	189	160 - 240 mPa.s
3. 2011-0461102-96 Acidität	76	MAX 200 ppm

Translation:

Pruefungen:	determinations
Ergebnisse:	results
Spezifikation:	specification
NCO-Gehalt:	NCO-content
Viskosität:	viscosity
Acidität:	acidity

F 02

Appendix - Analytical Characterization of Test Atmosphere

BAYER AG
DEPARTMENT OF TOXICOLOGY
FRIEDRICH-EBERT-STR. 217-333
D-42096 WUPPERTAL

DESMODUR 44 V 20L

ANALYTICAL METHOD VALIDATION FOR

CONCENTRATION DETERMINATIONS IN TEST ATMOSPHERES

Analytical Report

Dr. W. Rüngeler

Study-No.: T7062289

1. TABLE OF CONTENTS

2. SUMMARY

An analytical method was described that can be used to determine the concentration of the test material in test atmospheres and in diverse solutions.

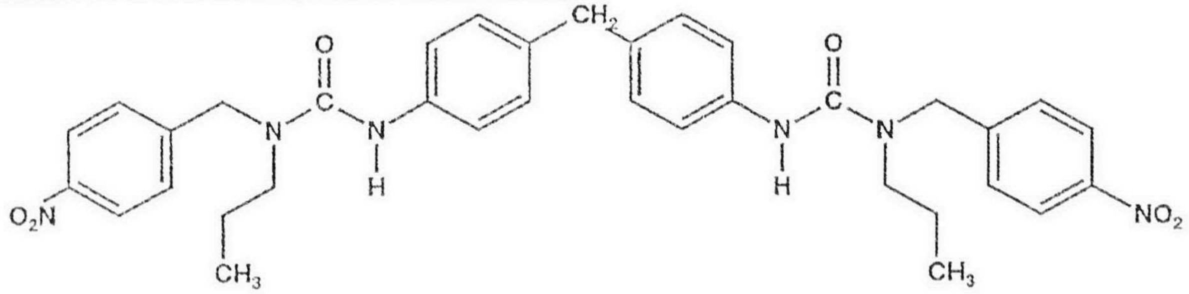
The test material as an aerosol was adsorbed on glass powder loaded with N-4-Nitrobenzyl-N-n-propylamine solution (nitro reagent). The isocyanate component reacted to form the corresponding urea derivative. After desorption with acetonitrile, the reaction product was quantified by high-performance liquid chromatography (HPLC; UV detection, 251 nm).
Standard solutions of the test material treated similary to test samples with the nitro reagent were used as basis for evaluation.
With a 50 litres atmosphere sample and an end solution volume of 10 ml, the limit of quantification for this test substance has been found to be **0.21 mg test material/m^3**.

For content checks in liquid application media the test material was placed in a solution of N-4-Nitrobenzyl-N-n-propylamine solution (nitro reagent). The isocyanate component reacted to form the corresponding urea derivative. After dilution with acetonitrile, the reaction product was quantified by high-performance liquid chromatography (HPLC; UV detection). Standard solutions of the test material treated similary to test samples with the nitro reagent were used as basis for evaluation.
The limit of quantification for this test substance has been found to be **1.06 µg test material/ml acetonitrile**.

3. INTRODUCTION

An analytical method for the quantification of DESMODUR 44 V 20L from test atmospheres was developed. This work was conducted in preparation for investigations on the inhalation toxicity of this test material. The method and its validation was described in this report.
In this method, developed by N. Kuck and modified by ourselves, the test material as an aerosol was allowed to react with N-4-nitrobenzyl-N-n-propylamine (nitro reagent) to form the corresponding urea compound (I), which was then determined by high-performance liquid chromatography (HPLC) with UV detection (251 nm). The test material aerosol was adsorbed from the test atmosphere in two series-connected tubes packed with glass powder loaded with the nitro reagent solution. The DESMODUR 44 V 20L urea derivative (I) was then desorbed with acetonitrile and the solution was injected, after appropriate dilution, onto the HPLC.
Standard solutions of the test material treated similary to test samples with the nitro-reagent were used as basis for evaluation.

DESMODUR 44 V 20L-urea derivative (I):



Investigations necessary for drafting the analytical method and performing analyses were conducted from January to March 1998 at the Department of Industrial Toxicology, Institute of Toxicology of Bayer AG, D-42096 Wuppertal-Elberfeld, Friedrich-Ebert-Strasse 217-333.
The study documentation (raw data and final analytical report) has been archived in locations specified by Bayer AG, in accordance with GLP requirements.

Study-No.: T7062289

The analytical method and its validation (HPLC) was presented in study no. T2060745 and was included in a separate report.

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4. MATERIALS AND METHOD

4.2. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

4.2.1. Apparatus

High performance liquid chromatograph HP1090 equipped with **
- Autosampler
- DAD (diode array detector)
- Integration: HP 3365 DOS-WorkStation/ChemServer **

supplied from Hewlett-Packard

4.2.2. Method

Column:	LiChrospher RP 18		5 µm; L: 125 mm; ID: 4mm; Grom	**
Oven temperature:			off	
Mobile phase:		A:	buffer solution	
		B:	acetonitrile	
		gradient program:	time 0 min: 50%B (start conditions)	
			time 3 min: 50%B	
			time 6 min. 85%B	
Flow rate:			1.0 ml/min	
Injection volume:			25.0 µl	
Detector:		wavelength:	251 nm	
		band width (BW):	4 nm	
		reference:	450 nm / 80 nm BW	**

4.3. OTHER APPARATUS

Gas measuring device (Elster) **
Mini A-Pump (P) (Leybold-Heräeus) **
Rotameter (R) **
Manometer (D) **
Needle valve (V)
calibrated thermometer
calibrated barometer
Standard laboratory equipment and glassware

small adsorption tubes with ground-glass joints (L = 120 mm, ID = 12 mm)
Packing: each tube 4 g glass powder
small adsorption tubes with ground-glass joints (L = 65 mm, ID = 12 mm)
Packing: each tube 2 g glass powder

Gas tight syringes (25 µl; 100 µl; 250 µl; 10 ml ; Hamilton) **
(The apparatus were regulary maintained and calibrated.)

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4.4. SOLVENTS AND CHEMICALS

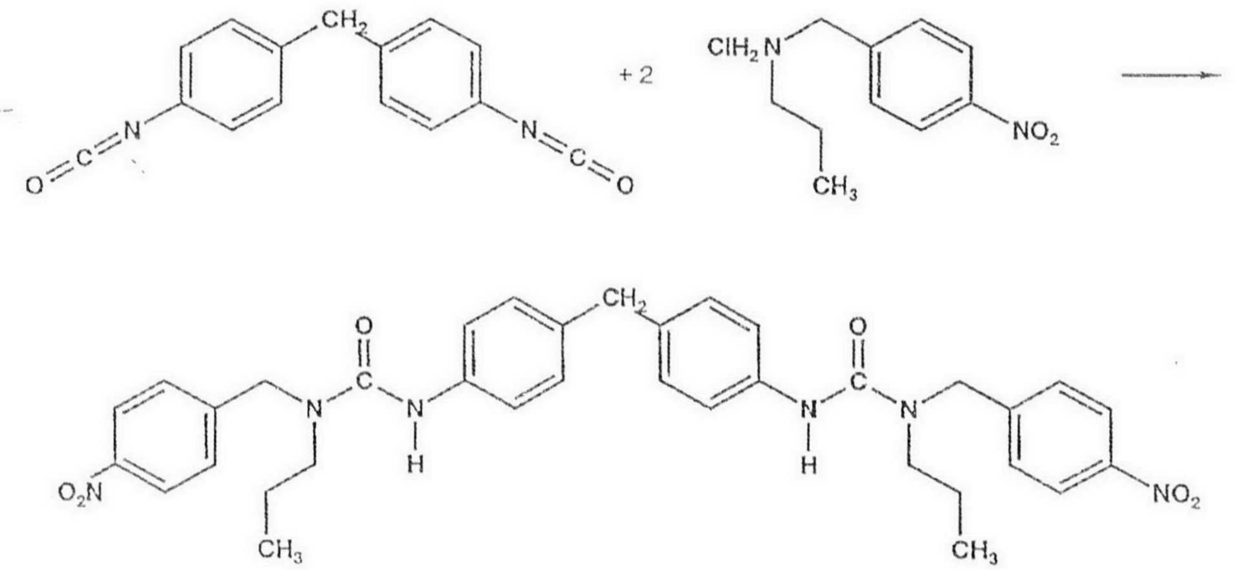
Ref. Material: DESMODUR 44 V 20L; Batch No. 7920/L2D; NCO-content: 31.5%; expiry date: April 22, 1998

Acetonitrile p.a.; Merck **
Deionized water (Milli-Q-water), Millipore unit **
Dichloromethane p.A., Merck **
N-4-Nitrobenzyl-N-n-propylammonium chloride p.A., Fa. Riedel de Haen, No. 33487 **
Glass powder 40/60 mesh; G. Karl, Part-No. GK 26-48004 **
Sodium sulfate p.A., Merck **
o-Phosphoric acid (85%ig); H_3PO_4 ; Merck **
Triethylamine (TEA) ; Merck **
Buffer composition: 3.5 ml H_3PO_4 + 4 ml TEA ad 1000 ml Milli-Q-water

** or equivalent

4.4.1. Nitro reagent solution (absorption solution)

3.2 g N-4-nitrobenzyl-N-n-propylammonium chloride (corresponding to 2.68 g free base) was dissolved in 200 ml {20 ml} of deionized water and 100 ml {10 ml} of 1 N sodium hydroxide solution was added. A white precipitate (free base) was formed. The aqueous suspension was transferred into an appropriate separating funnel and extracted with 250 ml {25 ml} dichloromethane twice. The organic phase was separated off, dried over sodium sulfate, transferred into a 1000 ml {100 ml} volumetric flask, and made up to the mark with dichloromethane. This solution contains 2.7 mg {27 mg} nitro reagent (free base)/ml dichloromethane. These solutions could be used as an absorption solution in impinger-flasks as well as for sample collection with glass powder-packed tubes, the nitro reagent serving to load the adsorbent carrier material. The used concentration for the chemisorption was presented in the raw data.



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empirical formula of the urea derivative: $C_{35}H_{33}N_6O_6$
The structure of the reaction product formed from DESMODUR 44 V 20L and nitro reagent was shown in the above equation. This urea derivative was analyzed in the HPLC (UV-detection) and was quantified as free DESMODUR 44 V 20L.

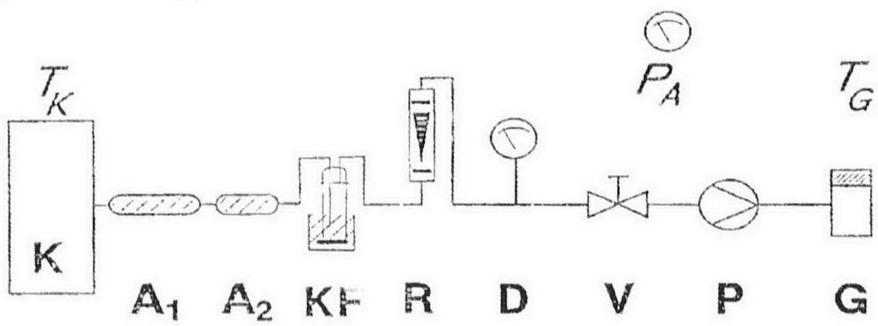
4.4.2. Calibration standards

Approximately 10-40 mg of the test material was pipetted into a 50 ml volumetric flask and accurately weighed. The flask was then brought up to volume with nitro reagent solution (concentration: 2.7 mg/ml). Comparison standards in the desired concentrations were prepared from this solution by dilution with acetonitrile.

5. SAMPLE COLLECTION AND PREPARATION

The surface of the glass powder in each adsorption tube was first loaded with 1 ml of the nitro reagent solution. The solvent was collected and discarded. Two series-connected adsorption tubes pretreated in the described way (A_1: 4g; A_2: 2g) were connected to the sampling apparatus (air throughput 0.5 to 1.0 l/min) (Fig. 1). The total volume of sampled air (V_X) the temperature of the gas flowmeter (T_G) the chamber temperature (T_K) and the barometric pressure (P_A) were recorded. After the end of the sample collection adsorption tubes (A_1, A_2) were mounted against the flow direction on an adequate volumetric flask. To desorb the urea derivative a funnel was fitted and approximately 75% of the end volume of acetonitrile was passed slowly through the tubes. The contents of the volumetric flask were then made up to the mark with acetonitrile. Samples of low concentration (approx. 1 mg/m³) were eluted with 10 ml of acetonitrile. Solutions were then injected onto the HPLC after appropriate dilution.

Figure 1: Sample collection apparatus



K	Inhalation chamber	V	Needle valve
A_1	Adsorption tube; packing: 4 g glass powder	P	Pump
A_2	Adsorption tube; packing: 2 g glass powder	T_G	Temperature of Gas flow meter
KF	condenser (optional)	T_K	Temperature of chamber
R	Rotameter	P_A	Barometric pressure
D	Manometer	G	Gas flow meter

6. CALIBRATION OF THE ANALYTICAL METHOD

To set up the calibration series, test material solutions in nitro reagent solution were prepared with appropriate concentrations (see 4.4.2.). Method-specific adjustments were made on the HPLC and 25.0 µl of each calibration concentration was injected for preparation of the calibration curve.

Measurement wavelength: 251 nm (see the UV spectrum, Fig. 2 pres. in study No. T2060745).

Fig. 3 shows a typical chromatogram of these external calibration solutions. A statistically evaluated calibration curve was shown in Fig. 4. This curve was plotted by the integrator and was based upon the injected concentrations. The calibration curve was plotted anew for each analysis sequence, and deviations from this calibration range were therefore possible. All sample concentrations were always within the calibration range documented for each sample sequence. The quantitative evaluation was performed by determination and comparing the peak area of **DESMODUR 44 V 20L urea derivative** of the analytical solution with the peak areas of the external standard solutions.

Retention time: **DESMODUR 44 V 20L urea derivative**
about 7.2 min **conc. range:** 1.06 to 21.2 µg/ml

Figure 2: UV spectra of the MDI urea derivative

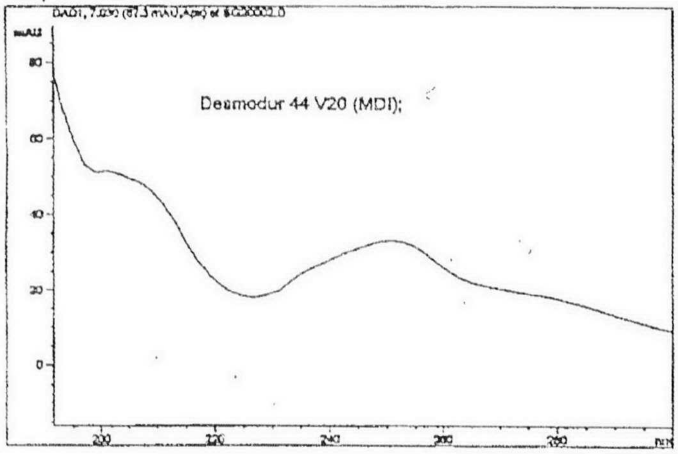


Figure 3.: typical LC-chromatogram of the test substance (calibration standard)
test material concentration: 10.56 µg/ml

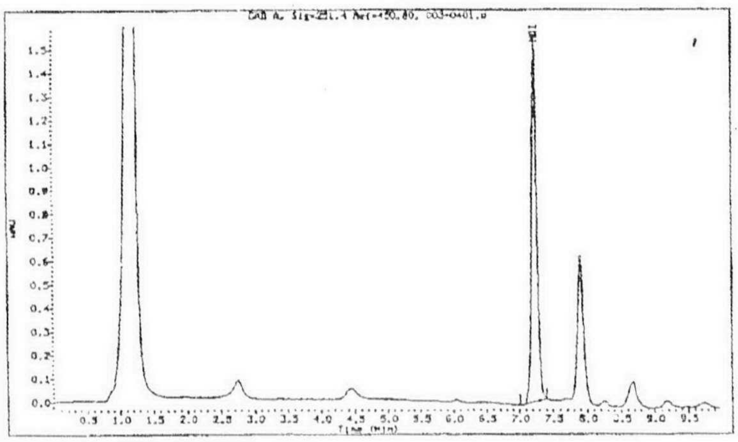
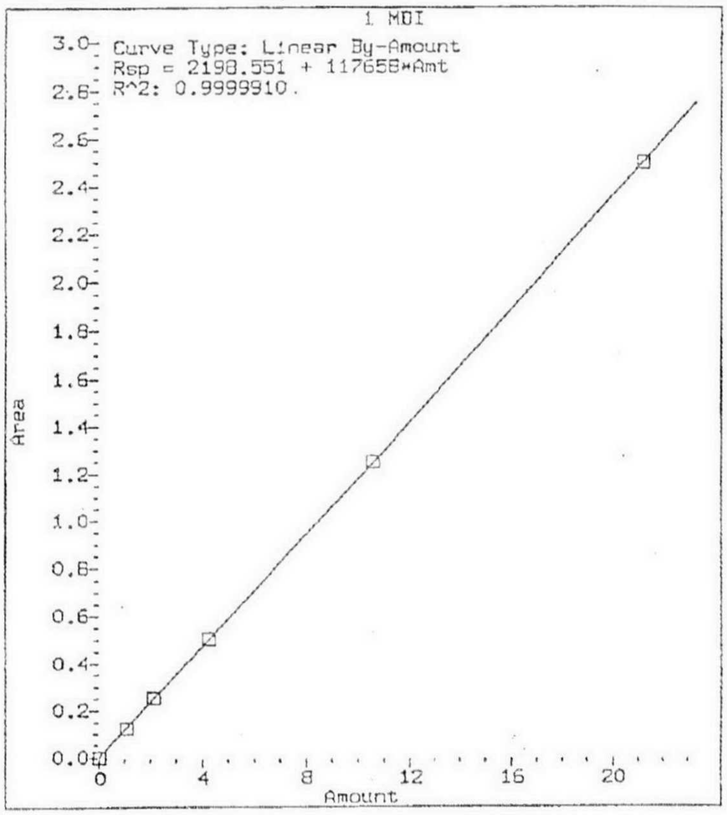


Figure 5: Calibration curve of the analytical method date: March 17, 1998



The calibration was linear in the range shown. The linear regression value was r^2 = 0.9999910.

7. CALCULATION OF THE ANALYTICAL RESULTS

Each sample within a sequence was injected twice. Since the sample and standard were treated identically, the concentration results do not need to be recalculated. The integrator evaluated each sample based on the plotted external standard calibration curve (see section 6.). The results were expressed in units of µg test material/ml solution.
The test material concentration in the test atmosphere was determined from the relationship:

$$\text{mg test material} / \text{m}^3 \text{ air} = \frac{X * F}{V_X}$$

F		dilution factor (e.g. 10/25 for undiluted analytical solution)
X	[µg/ml]	test material concentration in the analytical solution
V_x	[l]	chamber atmosphere collected volume

8. STABILITY

The stability of DESMODUR 44 V 20L urea derivative in acetonitrile and dichloromethane was checked at room temperature over a period of 5/6 days [study no. T2060745]. All solutions tested were found to be stable. No decrease in concentration was observed. The chromatographic sample preparation (elution of test material from glass powder, dilution, and injection) all were conducted during the tested time frames.

9. PRECISION

The precision of this analytical method was assessed by 10/9 separate injections for each of three relevant concentrations of the calibration standards. The area values obtained were presented in Table 1 (data presented in study no. T2060745). The precision of this method was found to satisfy the analytical requirements.

Table 1:

1.300 [µg/ml]	103.600 [µg/ml]
1.197	102.153
1.186	102.642
1.139	101.816
1.202	101.648
1.169	99.649
1.235	98.492
1.220	100.241
1.235	100.188
1.209	99.713
	99.559
MEAN = 1.199	MEAN = 100.615
c_V = 2.6%	c_V = 1.4%

10. DETECTION LIMIT

The limit of quantitation using this analytical method was 1.056 µg test material/ml in acetonitrile. With a sample collection volume of 50 litres and an end dilution volume of 10 ml, a concentration of **0.21 mg DESMODUR 44 V 20L/m^3** could be accurately determined.

11. LITERATURE

K.L. DUNLAP, R.L.SANDRIDGE, J.KELLER
Anal. Chem. 1976, 48, S. 497

R.L.SANDRIDGE, J.KELLER
Anal. Chem. 1979, 51, S. 1868

W. Rüngeler
Analytical Study Report (T2060745)

ACGIH (American Conference of Governmental Industrial Hygienists)

Air Sampling Instruments for Evaluation of Atmospheric Contaminants, 5th Edition, ACGIH (1978)

ChemG
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Bundesgesetzblatt, Part I of the 29th of July 1994

End of Report

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